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THE UNIVERSITY OF ALBERTA

AUXOTROPHIC MUTANTS OF THE SECOND CHROMOSOME OF *DROSOPHILA MELANOGASTER*

by

©

FARDOS NAGUIB MOHAMMED NAGUIB

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Auxotrophic Mutants of the Second Chromosome of *Drosophila Melanogaster*, submitted by Fardos Naguib Mohammed Naguib, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Approximately 1,800 second chromosomes of *D. melanogaster* have been mutagenized and screened for the existence of auxotrophic mutants, especially those producing RNA-requirement on Sang's defined medium. A novel mutagenesis screen, employing "criss-crossing" of the second chromosome balanced lethals *Pm/mr* and SM5/*Sp*, was used.

Five putative auxotrophs were isolated. Two, *yea8-1* and *yea9-1*, found to map near *Tft* (2-53.2), are heat-sensitive and only responsive to yeast supplementation. The third, a "true" auxotroph, *ade2-1*, is linked to *Sp* (2-22.0). It responds to either inosine or adenosine but not to guanosine. It is suggested to be genetically impaired, either prior to inosinate formation or, with some reservations, in the branches of purine biosynthesis leading to *de novo* adenylate production. Two other auxotrophs, *pyr2-1* and *pyr2-2*, are alleles and were found to be lined to bw^D (2-104.5). They respond to ribosylated naturally occuring purines and pyrimidines. In contrast, no response was observed with the corresponding unribosylated derivatives and with pyrimidine precursors. Enzyme assays showed excess orotate phosphoribosyl-transferase activity in these mutants. It is suggested that *pyr2-1* and *pyr2-2* are partially defective in phosphoribosyl pyrophosphate biosynthesis. Alternatively, they may be regulatory mutants affecting the pyrimidine biosynthesis pathway.

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LIST OF ABREVIATIONS

A	adenine
AMP	adenylate
APRT	adenine phosphoribosyltransferase
AR	adenosine
ATC	aspartate transcarbamylase:carbamyl aspartate transferase
C	cytosine
Carb. asp	carbamyl aspartate
Carb. phos	carbamyl phosphate
CdR	deoxycytidine
CMP	Cytidylate
CPSase	Carbamylphosphate synthetase
CR	cytidine
DHO	Dihydrooratate
DHOase	Dihydrooratase
DHO dehase	Dihydrooratate dehydrogenase
dCMP	deoxycytidylate
dTMP	deoxythymidylate
dUMP	deoxyuridylate
EMS	ethyl methanesulphonate
G	guanine
GMP	guanylate
GR	guanosine
H	hypoxanthine
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HR	Inosine

IMP	Inosinate
O	orotate
ODC ase	orotodylate decarboxylase
OMP	orotodylate
OPRTase	orotate phosphoribosyltransferase
OR	orotidine
P_i	inorganic phosphate
PP_i	pyrophosphate
PPRT	pyrimidine phosphoribosyltransferase
PR-	phosphoribosyl-
PRPP	PP-Ribose -P: phosphoribosyl pyrophosphate
RNA	ribonucleic acid
S'AMP	adenylo-succinate
T	thymine
TdR	thymidine
U	uracil
UdR	deoxyuridine
UMP	uridylate
UR	uridine
X	xanthine
XMP	xanthylate
XR	xanthosine

INTRODUCTION

AUXOTROPHY IN *DROSOPHILA MELANOGASTER*

For a number of years, a wide range of approaches has been made towards resolving the mechanism by which gene action is regulated in higher organisms. Auxotrophy was a major genetic tool behind the solution of the problem in microorganisms. Development of defined growth medium for *Drosophila melanogaster* (Sang, 1956) opened up the theroretical possibility of similar studies in a multicellular animal. However, whenever attempts were made at investigation of auxotrophy, the phenomenon seemed elusive. In the last decade, however, several breakthroughs have been made (Vyse and Nash, 1969; Norby, 1970; Falk and Nash, 1974) and what seemed unattainable is now a well-established research field (see, for example, Rawls and Fristrom, 1975).

Although some fruit-fly characteristics were known that exhibited nutritionally modifiable morphological defects, such as *antennaeless* (Gordon and Sang, 1941; Begg and Sang, 1945; Gordon, 1959), melanotic tumor production and suppression (Burnet and Sang, 1964; Sang and Burnet, 1967; Sparrow and Sang, 1974), *Bar* (Kaji, 1954, 1955; De Marinis, 1966 a and b; Fristrom, 1969) and *vermillion* (Tatum and Haagen-Smit, 1941; Baglioni, 1960). Systematic induction of auxotrophs was started only in 1969 (Vyse and Nash, 1969). This line of research has been extended by Falk and Nash (1974 a and b) Naguib and Nash (submitted for publication) and Nash (unpublished). Four main factors were behind this success:

(1) The availability of EMS, a chemical mutagen potent in inducing point mutations, but neither excessively toxic nor active as a

sterilant.

(2) The availability of a chemically defined medium on which *Drosophila* could grow axenically (Sang, 1956).

(3) The availability of *Drosophila melanogaster*, itself, which happens to be easily amenable to genetic manipulations; in addition, it is backed up by a huge body of lieterature touching on almost every aspect of its life cycle.

(4) The choice of nucleotide metabolism as a field for *Drosophila* auxotrophic studies, since the chemical interconversions involved are fairly well established and since *de novo* nucleotide biosynthesis is operative, as evidenced by lack of wild-type nucleoside requirements. A block in the *de novo* biosynthesis should lead to auxotrophy, given the presence of salvage pathways.

The body of this thesis concerns studies on new, autosomally inherited nucleoside-requiring auxotrophic mutants of the fruit fly. Reviews and compendia on the biology, genetics and biochemistry of *Drosophila melanogaster* can be found in Demerec (1950), Lindsley and Grell (1968), Fristrom (1970), Postlethwait and Schneiderman (1973), Dickinson and Sullivan (1975), Novitski and Ashburner (in press) and Wright and Ashburner (in preparation).

NUCLEOTIDE METABOLISM

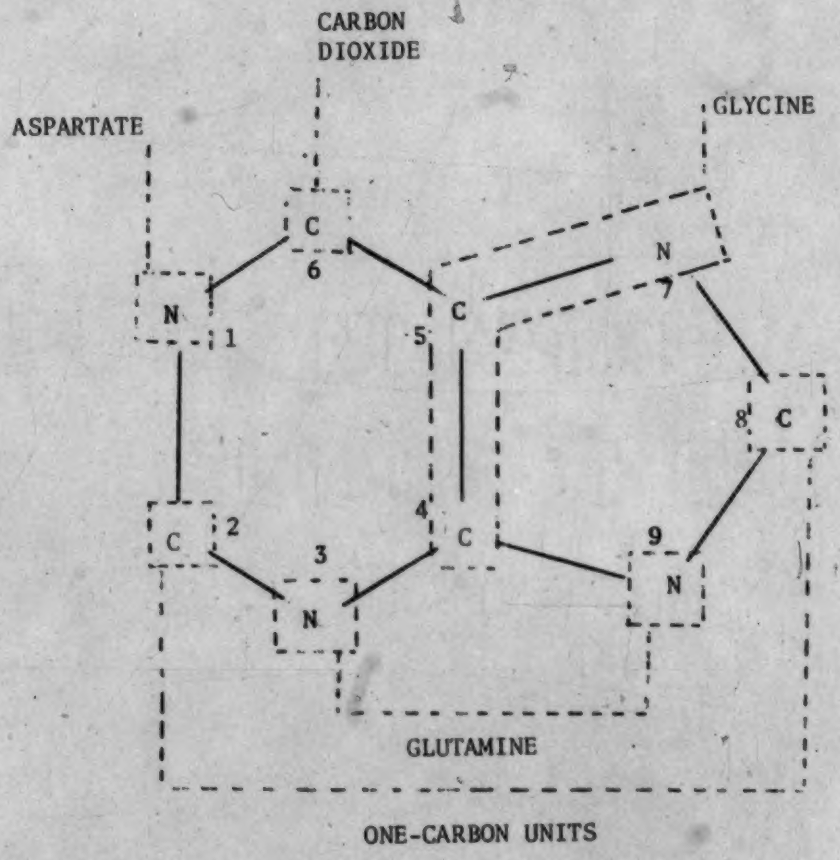
The following introduction surveys the historical development of our knowledge of the genetic basis of nucleotide metabolism. Nucleotides can be divided into two main classes: purine nucleotides and pyrimidine nucleotides. The systematic structural chemistry of these compounds is reviewed in Henderson and Paterson (1973).

Purine nucleotides

Initial evidence for the occurence of *de novo* biosynthesis of purine nucleotides came from growth of mammals on low purine diets that could not in any way account for the observed amount of growth (Socin, 1891). Studies with labelled compounds (1946-1951) led to the determination of the origin of each atom within the purine ring (Christman, 1952; Buchanan, 1958; Hartman and Buchanan, 1959); the basic structure of the purine ring is shown in Figure 1. These studies were followed (1951-1959) by the identification of the enzymes of purines biosynthesis *de novo* from pigeon and chicken liver (Buchanan, 1959; Hartman and Buchanan, 1959; Hartman, 1970; Henderson, 1972; Henderson and Paterson, 1973). Prior to, and along with, the identification of the enzymes of purine biosynthesis *de novo*, genetic blocks caused by auxotrophic mutations isolated in Enterobacteriaceae,yeast and certain other fungi have been used to establish or confirm the biosynthetic pathways of purines.

Initial experiments involved the use of nucleic acids or their derivatives as growth substances for microorganisms. It soon became evident that certain derivatives were "essential" or rate limiting for the growth of certain microorganisms. For example, Möller (1939) as reported by Pennington, demonstrated that adenine is required for the growth of *Streptobacterium plantarum*, whilst Pappenheimer and Hottle (1940) found adenine to be necessary for the growth of a strain of group A hemolytic streptococci. In the latter case, hypoxathine, guanine, xanthine, guanylic acid or adenylic acid could replace adenine. Snell and Mitchell (1941) showed that guanine is essential for the growth of *Leuconostoc mesenteroides*.

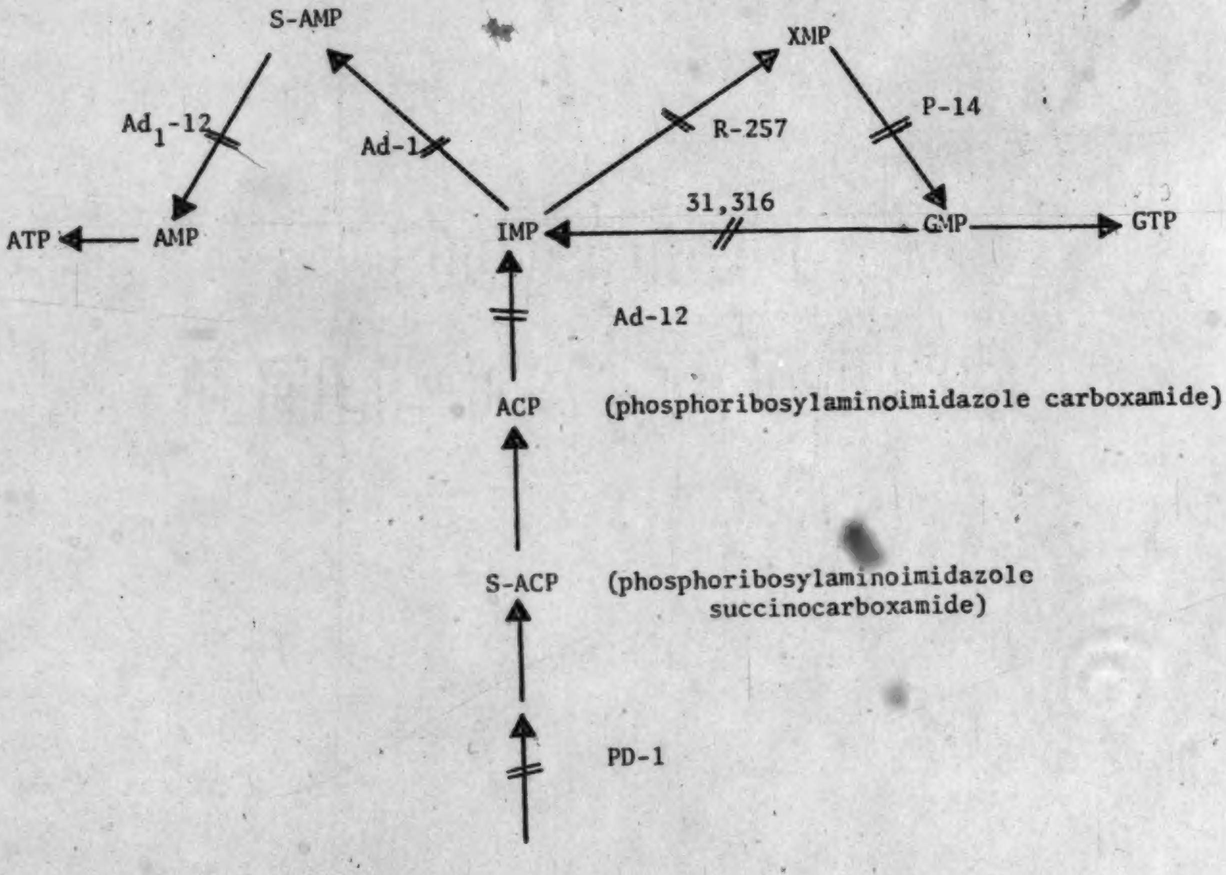
Figure 1 . The biosynthetic origins of the atoms constituting the ring structures in purines (Henderson, 1972).


CARBON
DIOXIDE
ASPARTATE
GLYCINE
C
6
N
1
C
5
N
7
8
C
C
2
3
N
4
C
9
N
GLUTAMINE
ONE-CARBON UNITS

Robbins and Kavanagh (1942) described the requirements of *Phycomyces* for guanine and hypoxanthine. Pennington (1942) reported the effects of adenine, guanine, and hypoxanthine on *Spirillum serpens* with, for the first time, emphasis on the inhibitory effects of certain purines on physiological activity of others. Along these lines, Elion and Hitchings (1950) found that *Lactobacillus casei*, known to be incapable of carrying out folic acid biosynthesis, could grow in the presence of thymine and any of several purines. They concluded that in this organism guanine could be converted to adenine and *vice versa*.

Meanwhile, as early as 1941, Beadle and Tatum were arguing that to study how genes regulate "the development and functioning of an organism, which essentially consist of an integrated system of chemical reactions" one should investigate when and how genes regulate known chemical reactions, rather than attempting to solve the chemical basis of known genetic characters. In other words, one should induce and use what are presently called auxotrophs to unravel the relationship between genes and biochemical pathways. In fact, in the same paper Beadle and Tatum, using X-rays, had already isolated tryptophanless, para-aminobenzoic - acidless, nicotinic-acidless and several other biochemical mutants (auxotrophs) in *Neurospora crassa*, among which one turned out to be the first adenineless mutant (Pierce and Loring, 1945; and Mitchell and Houlahan, 1946).

Roepke (1946) reported the isolation of purine-requiring mutants in *Escherichia coli*. Fries (1947-1948) followed with the first guanineless mutant, isolated in *Ophiostoma multiannulatum*. The first mutants characterized as representing blocks in the *de novo* purine biosynthesis pathway prior to inosinate (IMP) formation were reported in *E. coli* by Davis (1949) and Gots (1950). Abrams (1951) isolated an adenine requiring mutant in *Saccharomyces cerevisiae* that could also grow on hypoxanthine. In 1952, Ushiba and Magasanik isolated a guanineless mutant (*P14*) in *Aerobacter aerogenes* which was found later to accumulate xanthosine (Magasanik and Brooke,1954). They also isolated another mutant (*PD-1*) in *A. aerogenes* that they described as blocked in purine biosynthesis *de novo* prior to IMP formation. In 1956, Whitfeld (in *N. crassa*) and in 1957 Gots and Gollub (in *E. coli*) isolated adenylosuccinate lyase (then known as adenylosuccinase) defective mutants with which the latter confirmed the involvement of this enzyme in two of the twelve steps required for adenylate biosynthesis *de novo*. In 1957, Magasanik *et. al.*, and Moyed and Magasanik, using mutant *E. coli, A. aerogenes* and *Salmonella typhimurium* strains, confirmed the sequential conversion of inosinate to xanthylate then guanylate in the process of guanylate biosynthesis from inosinate. In the same year, Magasanik, in a review article, concluded that purine-requiring mutants isolated so far could be classified into four main groups: group 1 comprises those mutants which can grow on any of the four common, naturally occuring purines (adenine, guanine, xanthine and hypoxanthine); group 2 consists of those mutants that utilize xanthine or guanine but not hypoxanthine or adenine; group 3 is made of mutants with a specific requirement for guanine and group 4 of those with specific requirement for adenine. With the identification of guanylate reductase in *E. coli* (Mager and Magasanik, 1960) and the isolation of GMP-reductase defective mutants, the picture of purine nucleotide biosynthesis, salvage utilization and interconversion emerged as in the following diagram (modified from Magasanik and Karibian, 1960); the diagram represents a survey of the state of knowledge of purine biosynthesis in 1960 and includes all the mutant blocks known from bacteria at that time. For a more complete picture of the pathway, see Fig. 2 and 3.


S-AMP
XMP
Ad_1-12
Ad-1
R-257
P-14
31,316
ATP
AMP
IMP
GMP
GTP
Ad-12
ACP
(phosphoribosylaminoimidazole carboxamide)
S-ACP
(phosphoribosylaminoimidazole succinocarboxamide)
PD-1

Mutants 31 and 316 are not auxotrophic. They would be expected to grow on IMP and AMP but not on GMP in circumstances where *de novo* biosynthesis is blocked.

A year later, Levin and Magasanik (1961) reported for the first time enzyme repression in purine biosynthesis. In their study, they followed the specific activity of phosphoribosyl aminoimidazole carboxamide formyltransferase, inosinate cyclohydrase (inosinicase) and inosinate dehydrogenase in adenine, guanine and purine requiring mutants of *E. coli, S. typhimurium* and *A. aerogenes,* when grown on media with and without adenine and guanine at growth limiting and at excess concentrations. Nierlich and Magasanik (1965 a, b) extended the enzyme repression studies to phosphoribosyl amidotransferase, phosphoribosyl formylglycineamide synthetase and phosphoribosyl glycinamide synthetase of *A. aerogenes.* Momose *et. al.*, (1966, 1967) working with *B. subtilis*, extended the phenomenon to adenylosuccinate synthetase and adenylosuccinate lyase.

Clearly, the enzymes of purine biosynthesis *de novo* are subject to general repression under conditions of purine sufficiency and become derepressed in mutants subjected to limiting supplementation with purines.

In its initial phases, the use of genetic blocks in the investigation of purine metabolism vied with the use of "inhibition analysis". This approach crystallized in the late 40's and early 50's as a result of observations that substrates of genetically impaired enzymes tend to accumulate in the medium (Horowitz, 1946) and that analogues of a metabolite tend to inhibit the enzymatic conversion of that metabolite (see Shive, 1951). Inhibition analysis consists mainly in growing a given organism in the presence of an analogue, thus blocking the catalytic activity of a specific enzyme, then investigating metabolite accumulation by this organism. In the absence of genetic blocks, which were not easily obtainable until the development of fairly sophisticated selection techniques, inhibition analysis proved rewarding. For example, Shive *et. al.*, (1947) observed the accumulation of aminoimidazole carboxamide in *E. coli* sulfanilamide inhibited cells and concluded that it is a precursor of purines. Gots (1950) and Shive (1951) using the same system to which they added one or the other purine, reported that purines relieve the accumulation of the carboxamide derivatives. This result is clearly interpretable as a manifestation of regulation in the *de novo* pathway, although the authors did not view it as such. With increased induction of auxotrophic mutations in the late 50's and early 60's, inhibition analysis as an independent approach to the investigation of purine metabolism waned. However, in 1957, Gots described how purine end-products specifically inhibit the synthesis of the imidazole nucleus in an *E. coli* mutant blocked in the conversion of aminoimidazole carboxamide. In this context, the term "feedback inhibition" was applied for the first time to purine metabolism. This finding together with the demonstration by Wyngaarden and Ashton (1959) in pigeon liver that the first irreversible step in purine biosynthesis is the site of feed-back inhibition by end-products, triggered the investigation of end-product inhibition by purines. Because of the lack of mutations in purine biosynthesis in animal cells, a modification of inhibition analysis was applied to the problem of regulation. Thus LePage and Jones (1961) reported accumulation of reduced amounts of ^{14}C-formylglycinamide in aza-serine-inhibited-Erlich ascites tumor cells in the presence of adenine, guanine, hypoxanthine and those purinethiols (such as 6-thio-guanine and 6-mercaptopurine) that could be converted to nucleotides and, presumably, act as feed-back inhibitors. Henderson, (1962) observed the same phenomenon *in vitro* in the presence of adenine, hypoxanthine, guanine and aminoimidazole carboxamide.

Studies on bacteriostatic agents led to the conclusion that many were effective because they are analogues of normal metabolites. For example, the sulphanilamides (whose effects in blocking purine biosynthesis were outlined above) were shown by Woods (1940) and Fiddles (1940) to be para-amino-benzoic acid analogues. Para-amino-benzoic acid is a precursor of folate which is, in turn, necessary as a formyl donor in purine biosynthesis as well as other processes such as thiamine biosynthesis (see Henderson, 1972). Analogues of purines were studied systematically by Kidder and Dewey (1949), who classified them on the basis of their ability to support growth, to kill and, when toxic, to be neutralized by guanine, in *Tetrahymena geleii*. *T. geleii* was chosen as a test organism because it requires exogenous guanine, guanosine or guanylate for growth. Tavlitzki (1951) concluded that, in *S. cerevisiae* mutants defective in late *de novo* purine biosynthesis, growth stimulating purines also produce end-product inhibition, inferring that only those analogues that can be converted to nucleotides can produce end-product inhibition. At this point analogue resistant mutants were introduced as materials for study of purine metabolism. An early example of such a study by Elion *et. al.*, (1953) showed that a diaminopurine-resistant strain of *L. casei* lacked normal capacity to incorporate adenine into nucleic acids. Law (1956) and Anderson and Law (1960) reviewed this field extensively and showed that analogue resistance was commonly correlated with altered enzymatic activity. For example, 2-diaminopurine resistance was commonly associated with adenine phosphoribosyl-transferase (pyrophosphorylase) defects. It soon became clear that resistance to analogues could be used as a selective technique for isolating genetic blocks in biochemical pathways. Indeed, Kallé and Gots (1961) used resistance to diaminopurine to isolate purine phosphoribosyl-transferase deficient mutants. Lomax and Woods (1969) used sensitivity to diaminopurine to isolate a mutant with increased purine phosphoribosyl-transferase activity (three times the wild-type) in *S. cerevisiae*, which is normally resistant to this analogue. In 1961, Moyed had suggested "mutants selected for resistance might produce an enzyme with altered sensitivity to inhibition by the analogue as well as by the corresponding end product. Such mutants should be extremely useful for studying relationships between end-product inhibition and repression and for assessing the relative role of each process in the economy of the cell". In 1965, Momose *et. al.*, selecting for guanosine resistance, isolated "derepressed" strains of *B. subtilis*, namely GR-40 and GR-75. These exhibited a derepression of inosinate transformylase, yet repression was observed when the mutants were grown in the presence of adenosine and guanosine. It was suggested that the mutants must be altered in the regulator or operator of IMP-transformylase especially since test with IMP-dehydrogenase did not show any coordinate derepression with the IMP-transformylase. Heslot *et. al.*, (1966) and Nagy (1970) reported on a *6-azaguanine* resistant mutant in *Schizosaccharomyces pombe*. This mutant had a regulatory defect of the first enzyme in purine biosynthesis (phosphoribosyl amidotransferase), such that it had lost the capacity to be feed-back inhibited.

Thus, there have been four major lines of study, apart from straight biochemistry, that have led us to our present knowledge of purine biosynthesis and its control. For a more complete review of the subject see Henderson (1972). A series of reviews and catalogues of purine (and other) mutants in microorganisms is available; in *S. typhimurium* (Gots *et. al.*, 1969; Sanderson, 1970); in *E. coli* (Taylor and Trotter, 1967; Tritz *et. al.*, 1970); in *S. pombe* (Gutz, *et. al.*, 1975); in *S. cerevisiae* (Plischke, *et. al.*, 1975) and in *N. crassa* (Lakshimi and Wellman, 1975). The reader is referred to these for detailed references to the many mutants now known.

Purine mutants in higher organisms are less common; in humans, Seegmiller *et. al.* (1968) related the Lesch-Nyhan syndrome to a deficiency in hypoxanthine-guanine phosphoribosyl-transferase. Howell *et. al.* (1962) related gout to glucose-6-phosphatase deficiency, which presumably resulted in an increase in the endogenous phosphoribosyl pyrophosphate level, leading to an accelerated rate of purine biosynthesis *de novo*. Discovery of other cases of gout in which the primary mutant defect was increased activity of PRPP-synthetase (Becker *et. al.* 1975) confirmed the relation between glucose-6-phosphatase deficiency and gout.

In *Drosophila*, Shultz and Mann (1951) reported the first RNA-supplementable strain which they showed to grow satisfactorily when AMP was substituted for RNA. In the same year, Hinton *et. al.* (1951) characterized a strain containing *Inversion (2LR) 40d* as an adenine requirer. In 1952, Hinton and Roberts described two adenine-requiring strains, one of which seemed to have a dominant requirement. In 1959, Hinton detected further strains with requirements for adenine. None of the above requirements could be ascribed to specific gene loci. In 1969, Vyse and Nash undertook a systematic search for RNA-requiring mutants and succeeded in isolating two requirers, one of which, *1308*, exhibited a double requirement for purines and pyrimidines (Vyse and Sang, 1971). Falk (1973), modified and extended Vyse and Nash's approach (1969), isolating four purine requiring mutants; two (*pur1-1* and *pur1-2*) were alleles that responded to either adenosine or guanosine. *pur1-2* exhibited a markedly better response to adenosine; *ade1-1*^{sd}, which was slow developing on nucleoside-free medium, responded with normal growth rate to adenosine and was guanosine-sensitive; finally, *gua1-1*^{ts} required guanosine for its growth at 29°C.

A diagram of purine biosynthesis *de novo* up to IMP formation is shown in fig. 2 . This pathway is practically universal, although the regulation of each enzyme of the pathway may vary from one organism to the other. Production, interconversion, degradation and "salvage" pathways for the formation of adenylate and guanylate are shown in fig. 3 . *De novo* production of adenylate and guanylate is universal too. However, some microorganisms like *E. coli* and related Enterobacteriaceae can convert adenylate to guanylate and *vice versa*. Other microorganisms like *Torulopsis utilis* can convert adenylate to guanylate yet, the reverse conversion is not possible. Other microorganisms can only convert guanylate to adenylate as for example *T. geleii* and *L. leichmanii* (Brown, 1953). Animals generally have been thought only to convert adenylate to guanylate (see DISCUSSION). Moreover, synthesis of nucleotides from bases or nucleosides also show differences from one organism to the other.

Figure 2 . The pathway of inosinate biosynthesis *de novo*. This pathway is essentially similar in all species in which it has been studied. (From Henderson, 1972, with modification)

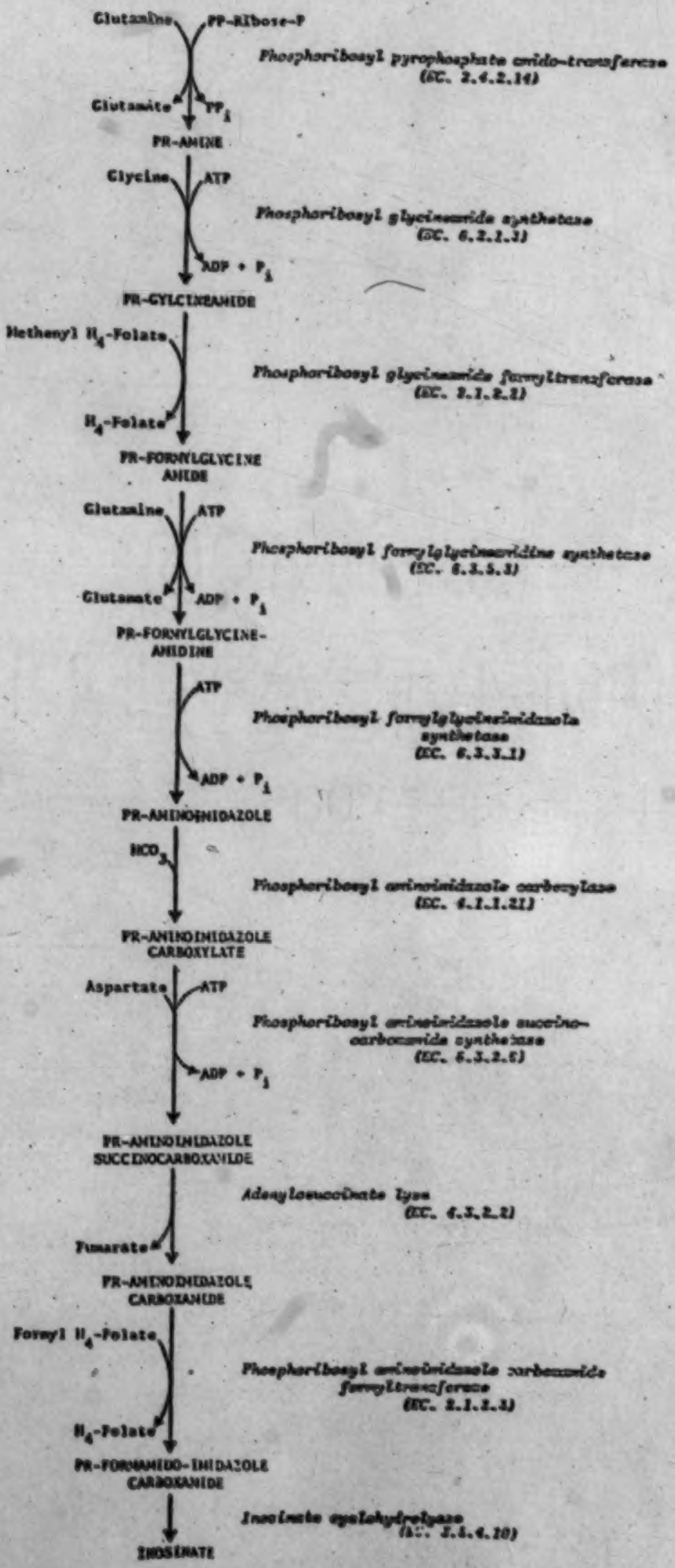
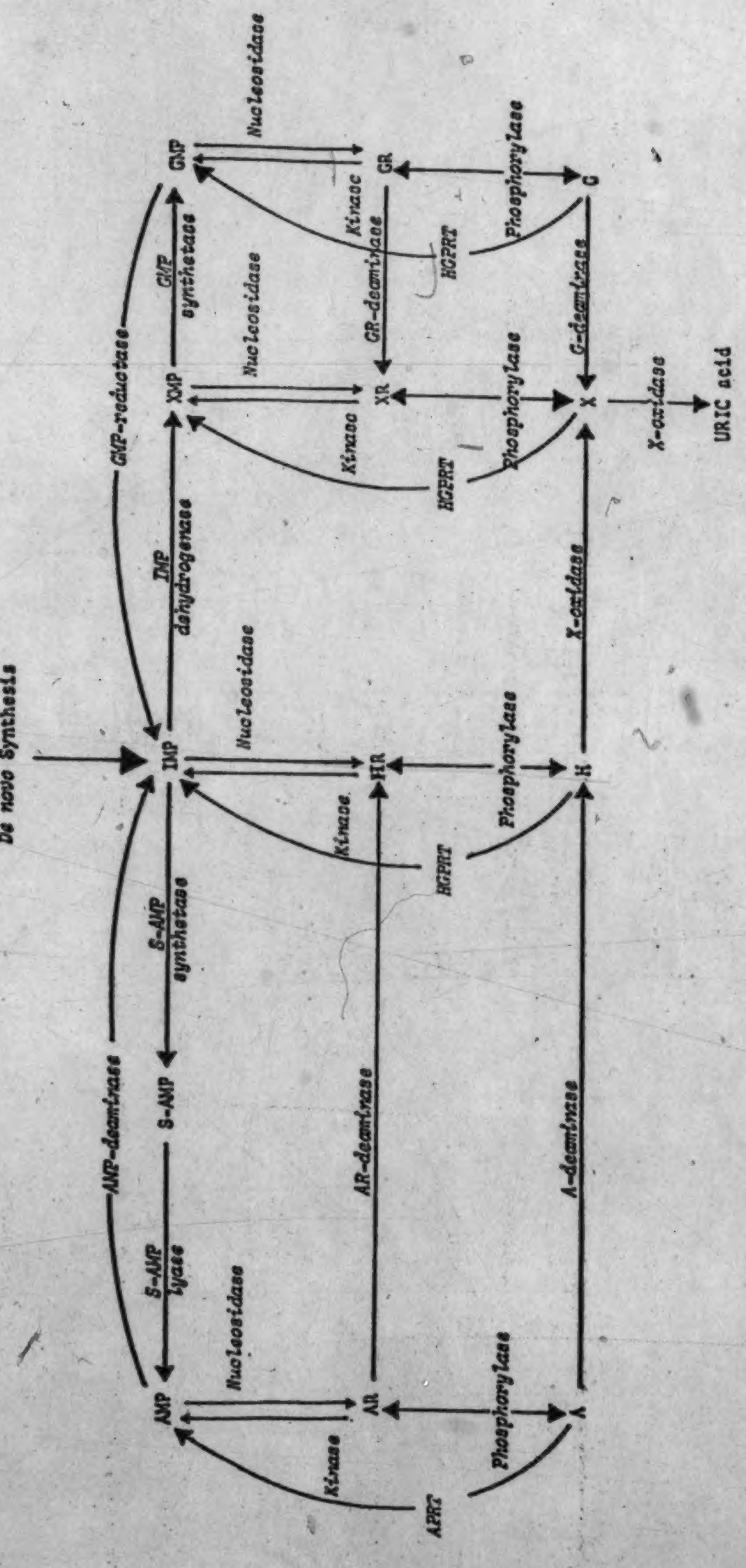

Glutamine
PP-Ribose-P
Phosphoribosyl pyrophosphate amido-transferase
(EC. 2.4.2.14)
Glutamate
PP_i
PR-AMINE
Glycine
ATP
Phosphoribosyl glycineamide synthetase
(EC. 6.3.1.3)
ADP + P_i
PR-GYLCINEAMIDE
Methenyl H_4-Folate
Phosphoribosyl glycineamide formyltransferase
(EC. 2.1.2.2)
H_4-Folate
PR-FORMYLGLYCINE AMIDE
Glutamine
ATP
Phosphoribosyl formylglycineamidine synthetase
(EC. 6.3.5.3)
Glutamate
ADP + P_i
PR-FORMYLGLYCINE-AMIDINE
ATP
Phosphoribosyl formylglycineimidazole synthetase
(EC. 6.3.3.1)
ADP + P_i
PR-AMINOIMIDAZOLE
HCO_3
Phosphoribosyl aminoimidazole carboxylase
(EC. 4.1.1.21)
PR-AMINOIMIDAZOLE CARBOXYLATE
Aspartate
ATP
Phosphoribosyl aminoimidazole succino-carboxamide synthetase
(EC. 6.3.2.6)
ADP + P_i
PR-AMINOIMIDAZOLE SUCCINOCARBOXAMIDE
Adenylosuccinate lyase
(EC. 4.3.2.2)
Fumarate
PR-AMINOIMIDAZOLE CARBOXAMIDE
Formyl H_4-Folate
Phosphoribosyl aminoimidazole carboxamide formyltransferase
(EC. 2.1.2.3)
H_4-Folate
PR-FORMAMIDO-IMIDAZOLE CARBOXAMIDE
Inosinate cyclohydrolase
(EC. 3.5.4.10)
INOSINATE

Figure 3 . The pathways of interconversion, degradation and salvage biosynthesis of purine nucleotides. Several of the enzymes shown are absent from certain species, as described in the text. (Miller and Collins, 1972; Becker, 1974 with modification).


De novo Synthesis
IMP
AMP
S-AMP
GMP
XMP
AR
HR
XR
GR
A
H
X
G
URIC acid
AMP-deaminase
GMP-reductase
S-AMP lyase
S-AMP synthetase
IMP dehydrogenase
GMP synthetase
Nucleosidase
Nucleosidase
Nucleosidase
Nucleosidase
Kinase
Kinase
Kinase
Kinase
AR-deaminase
GR-deaminase
Phosphorylase
Phosphorylase
Phosphorylase
Phosphorylase
Phosphorylase
Phosphorylase
Phosphorylase
Phosphorylase
APRT
HGPRT
HGPRT
HGPRT
A-deaminase
X-oxidase
X-oxidase
G-deaminase

For example, humans produce hypoxanthine-guanine phosphoribosyl-transferase; Miller and Collins (1972) using *Musca domestica* ovaries, and Becker (1974) using *D. melanogaster* tissue cultures, suggest the absence of this enzyme from these organisms. Nucleoside kinase also exhibits the same kind of heterogeneity. In contrast, the conversion of nucleotide monophospahtes to triphosphates is absolutely universal.

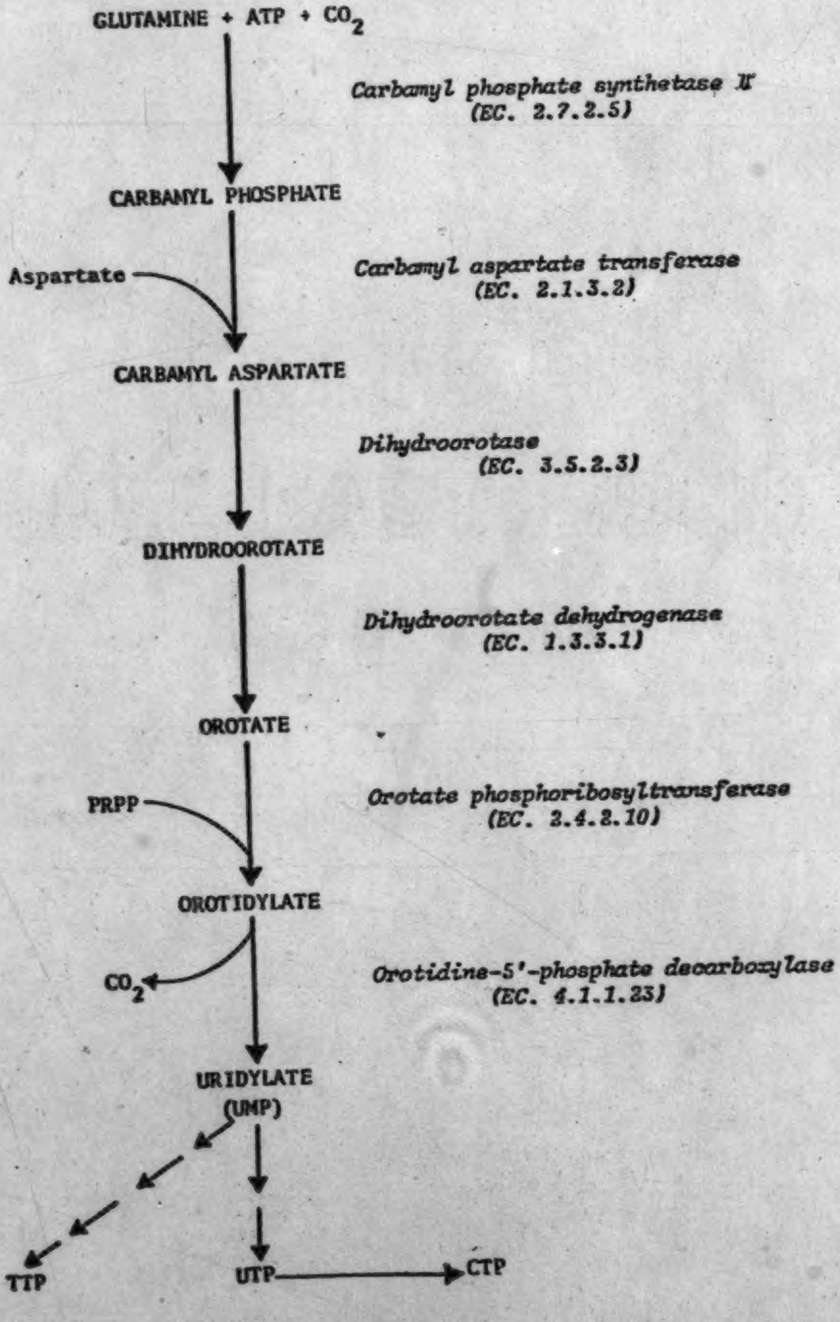
Pyrimidine Nucleotides

The historical development of pyrimidine metabolism followed more or less the same pattern as purines. Information concerning the genetic control and regulation of pyrimidine biosynthesis in microorganisms is extensively reviewed by O'Donovan and Neuhard (1970).

The pyrimidine pathway contains fewer steps than the purine pathway and, for the purpose of auxotrophic studies, can be considered "linear" (as opposed to the "bifurcated" purine pathway) with the final product, CTP, and UTP as the essential compounds formed. Production of thymidylate, the deoxy compound, is somewhat more complex than production of deoxycytidylate and the purine deoxyribotides. The reactions involved in pyrimidine biosynthesis are summarized in Fig. 4.

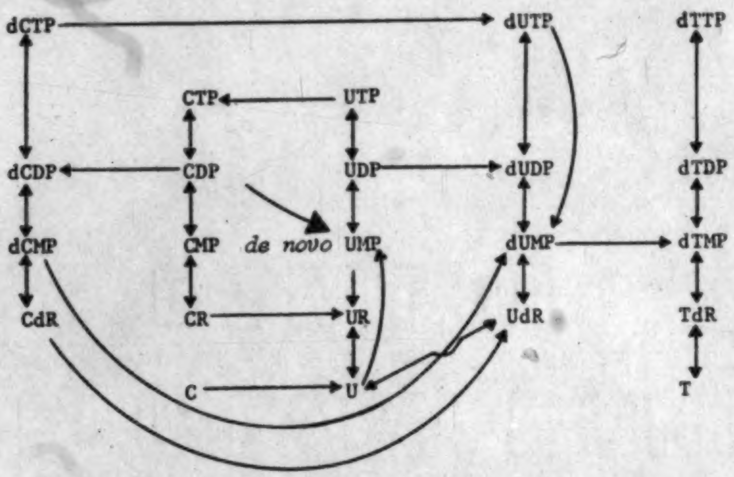
Auxotrophic mutants responsive to uridylate precursors (including cytidine and cytosine), to cytidine only and to thymidine precursors are known in various organisms. Bacterial mutants blocked in carbamyl phosphate synthetase (CPSase) require both pyrimidines and arginine (see below). Mutations in UMP-kinase or UDP-kinase are unconditionally lethal except when leaky. The leaky mutants do not exhibit pyrimidine-requirement but accumulate their substrates in the culture medium. They

Figure 4 . The orotate pathway of pyrimidine nucleotide biosynthesis *de novo*. This pathway is essentially similar in all organisms in which it has been studied. (Information derived from Henderson and Paterson, 1973).


GLUTAMINE + ATP + CO_2
Carbamyl phosphate synthetase II
(EC. 2.7.2.5)
CARBAMYL PHOSPHATE
Aspartate
Carbamyl aspartate transferase
(EC. 2.1.3.2)
CARBAMYL ASPARTATE
Dihydroorotase
(EC. 3.5.2.3)
DIHYDROOROTATE
Dihydroorotate dehydrogenase
(EC. 1.3.3.1)
OROTATE
PRPP
Orotate phosphoribosyltransferase
(EC. 2.4.2.10)
OROTIDYLATE
CO_2
Orotidine-5'-phosphate decarboxylase
(EC. 4.1.1.23)
URIDYLATE
(UMP)
TTP
UTP
CTP

are reported to mimic constitutive regulatory mutations affecting earlier enzymes of the pathway (Ingraham and Neuhard, 1972).

The pathways of interconversion and salvage utilization of pyrimidines are shown below.



Some variations would be expected in the characteristics of the mutants, depending upon the capacity of various organisms to utilize exogenous pyrimidine sources. For example, bacteria and yeast can convert cytosine to uracil, but animal cells cannot (Hahn and Lentzel, 1923; and Beck *et. al.*, 1972). Consequently, *rudimentary* mutants of *D. melanogaster*, which are blocked in early pyrimidine biosynthesis, do not grow on cytosine as they do on cytidine (Norby, 1970). Equivalent mutants in microorganisms grow on either cytidylate derivative. Yeast, in contrast to mammalian cells and bacteria, is not able to use exogenous thymidine since, in addition to the universal lack of thymine phosphoribosyl-transferase, it is devoid of thymidine kinase activity (Grivell and Jackson, 1968). Thymidine-requiring mutants, defective in thymidylate synthetase (thy$^-$) cannot, consequently, occur in yeast, unless as double mutants with mutants induced to incorporate TMP into their nucleic acids. In bacteria thy- mutants are reported (Okada *et. al.*, 1961, 1962). They are characterized by elevated levels of thymidine kinase, as can be expected in cases of starvation for TTP.

Enzymes in pyrimidine biosynthesis prior to UMP formation (see fig. 4) vary from one organism to the other, both with respect to the properties of individual enzymes and their regulation. In *E. coli* and *S. typhimurium*, one type of carbamyl phosphate synthethase (CPSase), which has dual substrate specificity for ammonia or glutamine is used in two different pathways, one concerned with arginine production and the other with pyrimidine biosynthesis (Abd-El-Al *et. al.*, 1969). Substrates of both pathways, as well as inosine, act as steric regulators of the catalytic activity of bacterial CPSase. In the case of fungi, it is found that two types of CPSase exist, one specific for arginine biosynthesis (CPSase I) and the other (CPSase II) for pyrimidine biosynthesis (Davis, 1961). It was also found that in these lower eukaryotes, CPSase II and the second enzyme of pyrimidine biosynthesis, aspartate transcarbamylase (ATCase) form an enzyme complex (Davis, 1960). In higher eukaryotes, the complex, which is found in the cytoplasm, also probably includes the third enzyme, dihydroorotase (Rawls and Fristrom, 1975; Jarry and Falk, 1974). In eukaryotes, CPSase is needed in pyrimidine biosynthesis, arginine biosynthesis (except in animals) and in the urea cycle of ureotelic organisms. Those higher eukaryotes that biosynthetize arginine might be expected to have two types of CPSase, one specific for arginine and the other for pyrimidine biosynthesis. Animals, which have an obligate dietary requirement for arginine, if they were ureotelic, would also be expected to synthetize two species of CPSase. This is indeed the case in mammals, where the mitochondria contain CPSase I and the cytoplasm of some tissues contains CPSase II (Henderson and Paterson, 1973). In contrast, if they were uricotelic, they should exhibit only one type of enzyme, CPSase II. Indeed in *Drosophila* only one type, CPSase II is present (Jarry and Falk, pers. comm.). The functional compartmentalization which is normal for the two CPSase enzymes is not necessarily absolute, since a double mutant defective in CPSase II and in ornithine transcarbamylase (in the arginine pathway) can be supplemented by arginine and one defective in CPSase I and ATCase can be supplemented by pyrimidines (Davis, 1967). A similar conclusion can be drawn for experiments on rat liver (Kesner, 1965). In higher eukaryotes, CPSase II is the first enzyme of the pyrimidine pathway. It is therefore expected to regulate pyrimidine biosynthesis *de novo*. In contrast to PRPP-amidotransferase,which regulates purine biosynthesis by being feed-back inhibited, (see section on purine nucleotides above), it would seem that CPSase exerts its regulatory effects by being rate limiting (Tatibana and Shigesada, 1972; Jarry and Falk, 1974), although it is also reported that it is feed-back inhibited by UTP (Tatibana and Ito, 1967). Moreover it has been demonstrated in human lymphocytes that CPSase is induced by phytohematoglutinin (Ito and Uchino, 1971). Whether this is a classical type of enzyme induction remains to be seen. In bacteria, on the other hand, CPSase is repressible.

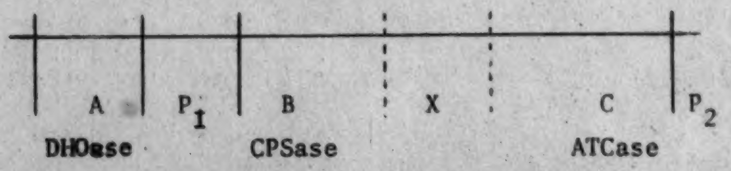
ATCase is the second enzyme of the pathway. In *E. coli, S. typhimurium* and related Enterobacteriaceae,it is the first enzyme unique to pyrimidine biosynthesis and might be expected to play a regulatory role; it is feed-back inhibited by CTP (Gerhart and Pardee, 1962). In eukaryotes, UTP acts as a feed-back inhibitor. However, it is reported that ATCase in these organisms is not in anyway rate limiting to pyrimidine biosynthesis (Hager and Jones, 1967; Tatibana and Ito, 1969). ATCase is reported to be repressible in bacteria (Yates and Pardee, 1957). In mammalian systems ATCase is derepressible and Roux *et. al.*, (1973) reported induction of the enzyme in mouse salivary glands.

Dihydroorotase (DHOase), dihydroorotate dehydrogenase (DHO dehase) and orotate phosphoribosyl-transferase (OPRTase) are reversible. DHOase and DHOdehase are not reported to play any significant regulatory role in the pathway. They seem to be repressible in Enterobacteriaceae and sequentially inducible in yeast (Lacroute, 1968) and fungi in general (O'Donovan and Neuhard, 1970).

OPRTase on the other hand is more thoroughly investigated. In yeast and bacteria it is specific for orotate (Dahl, *et. al.* 1959; Crawford, *et. al.*, 1957 and Lindsay *et. al.* 1972). In contrast, the same pyrimidine phosphoribosyl-transferase (PPRTase) seems to be responsible for the conversion of both orotate to orotidylate and uracil to uridylate in mammalian systems (Reyes and Guganig, 1975). As one of the substrates of OPRTase is PRPP, which was found to be rate limiting in purine biosynthesis (Henderson, 1972; Becker *et. al.* 1975) it is speculated that this enzyme may have regulatory effects on pyrimidine biosynthesis (Hoogenraad and Lee, 1974). It is reported that OPRTase is rate limiting in pyrimidine biosynthesis in Ehrlich ascites cells (Shoaf and Jones, 1973). Microorganisms starved for pyrimidines accumulate large amounts of orotate (Mitchell *et. al.*, 1948; Yates and Pardee, 1956) implying a possible derepression of, at least, the first four enzymes of the pathway, leading to rate limitation by OPRTase. In fact, Yates and Pardee (1957) showed that uracil starvation results in increased synthesis of all six enzymes of the *de novo* pyrimidine pathway, but Dennis and Herman (1970) showed that the synthesis of DHO dehase is not coordinate with synthesis of OPRTase. In *Drosophila*, elevated activity is reported for the fourth enzyme (DHO dehase) in *rudimentary* mutants, which are defective in the activities of one or more of the first three enzymes of pyrimidine biosynthesis (Rawls and Fristrom, 1975). In bacteria and yeast, OPRTase is not complexed to orotidylate decarboxylase (ODCase)(O'Donovan and Neuhard, 1970; Jund and Lacroute, 1972). In humans and other mammalian systems, on the other hand, it is reported that OPRTase and ODCase form an enzyme complex (Krooth, 1964; Brown *et. al.* 1972; Grobner and Kelley, 1975 and Reyes and Guganig, 1975). Defects in OPRTase are associated with the human disease, orotic aciduria and defects in ODCase with orotidinuria. In both bacteria and yeast, OPRTase is feedback inhibited by OMP (O'Donovan and Neuhard, 1970; Umezu *et. al.* 1971). ODCase on the other hand is inhibited by GMP in yeast and CMP in mammalian systems.

True regulatory mutants in pyrimidine biosynthesis *de novo* have not been isolated yet. All apparently constitutive mutants have so far turned out to be uridine kinase defects as described above.

From the viewpoint of the work included in this thesis, perhaps the most interesting development, in recent years, has been the elucidation of the sex-linked *rudimentary (r)* locus of *D. melanogaster*. *r* mutants are known to exhibit small wings and female sterility. In 1970, Norby demonstrated that *r* mutants require dietary pyrimidines. On the basis of their response to pyrimidine precursors, he concluded that *r* mutants must result from a block in one of the first two steps of pyrimidine biosynthesis. With the report that dietary pyrimidines restored female fertility to *r* mutants whilst they had no effect on the fertility of wild type flies (Bahn, 1970), it became definitive that the *r* locus is involved in pyrimdine biosynthesis. In 1973, Norby demonstrated biochemically that some *r* mutants have genetic blocks in aspartate transcarbamylase and suggested that carbamyl phosphate synthetase might possibly be involved too. In 1974, Jarry and Falk proved the involvement of carbamyl phosphate synthetase and suggested that *r* mutants may also be blocked in dihydroorotase. Rawls and Fristrom (1975) demonstrated that *r* mutants are defective in dihydroorotase activity. Prior to Norby's discovery of the nutritional requirement of *r* mutants, complementation studies had been carried out by Fahmy and Fahmy (1959), Green (1963) and Carlson (1971). The latter showed parallel complementation patterns with respect to wing defects and female sterility. Bahn *et. al.* (1971) extended this parallel to pyrimidine requirement. Carlson had concluded that the *r* locus is made of seven complementation units. Jarry and Falk simplified the situation to three main complementation units which map as three contiguous genetic regions as shown in the following diagram.



They believe that these regions (A, B and C) represent structural genes for the three enzymes. P_1 and P_2 contain alleles that do not complement with any other alleles and they suggest that one or other of P_1

and P_2 is regulatory for the entire locus.

The region X contains mutants which fail to complement with both B and C alleles. Falk (submitted for publication) has recently proposed that these mutants are in the region coding for the ATCase polypeptide which is necessary to stabilise CPSase in the enzyme complex.

That *r* is indeed the structural gene for these enzymes is supported by the finding that levels of enzyme activity are directly proportional to gene dosage within a given sex (Rawls and Fristrom, 1975) and that the molecular weight of the enzyme complex is reduced substantially in at least one presumptively CPSase$^-$ ATCase$^+$ DHOase$^+$ *rudimentary* mutant (Söderholm, *et. al.* 1975).

Clearly, more purine and pyrimidine-requiring mutants need be isolated and characterized if, nucleotide metabolism in *Drosophila*, as a representative of higher organisms, is to be elucidated. The present investigation was undertaken in this spirit.

MATERIAL AND METHODS

MATERIALS

Stocks

The various stocks used in the present study are described in Table 1.

The usefulness of isolating auxotrophs in a homogeneous genetic background was argued by Falk and Nash (1974b). It was initially decided to isolate autosomal auxotrophs in the same "Amherst" background as they used. To this end, an attempt was made to put the balanced lethal system *S M(2)S7* bw^D/ dp^{txI} *Cy* InsO *pr* cn^2 *sp* (Abrahamson and Meyer, 1965) into the "Amherst" genetic background, by backcrossing. However, this combination turned out to be lethal. The use of *Cy Xa/P, Ubx* system (Fig. 5) would have achieved a similar effect, as it would have yielded flies isogenic for all but the fourth chromosome, which presumably could be ignored. When this system was rejected, it was decided to ignore the background problem in order to save time. Nevertheless, the mutagenized chromosome was to be kept intact as can been seen in the scheme finally adopted (Fig. 6). This decision was based on the facts that:

(1) The second chromosome, bearing the mutants, originates from the Amherst stock.

(2) Any mutation isolated could easily be put in the "Amherst" background, by substituting first and third chromosomes, as long as the mutagenized chromosome itself was intact.

(3) There is no guarantee that the new balanced system would not be lethal in the "Amherst" background, especially since the bw^{V1}

Table 1: Description of Stocks

	Stock Designation			Description*	Source
1.	$AmOr^+$	wild type		Amherst Oregon	Amherst College Amherst, Mass.
2.	$mr\ bs^2/bw^{V1},ds^{33k}$	mr	:	morula (2-106.7)	California Institute of Technology (Cal. Tech.) Pasadena, Calif.
		bs^2	:	blistered (2-107.3)	
		bw^{V1}	:	brown-Variegated (2-104.5), also known as Plum (Pm)	
		ds^{33k}	:	dachsous (2-0.3)	
3.	$Sp/SM5,al^2,Cy,lt^v$ sp^2	Sp	:	Sternopleural (2-22.0)	Cal. Tech.
		SM5	:	most complete balancer for chromosome 2	
		Cy	:	Curly (2-6.1)	
		al^2	:	aristaless (2-0.01)	
		lt^v	:	light (2-55.)	
		sp^2	:	speck (2-107.0)	
4.	*pupal*	pu	:	pupal, isolated from mutagenized stock 1	University of Alberta (U.ofA.) Edmonton, Alberta
5.	$al\ dp\ b\ pr\ c\ px\ sp$	dp	:	dumpy (2-13.0)	Cal. Tech.
		b	:	black (2-48.5)	
		pr	:	purple (2-54.5)	
		c	:	curved (2-75.5)	
		px	:	plexus (2-100.5)	
6.	bw^D	bw^D	:	brown Dominant (2-104.5)	Cal. Tech.
7.	$J/In\ (2L)\ NS$	J	:	Jammed (2-41.0)	Cal. Tech.
8.	$S\ Sp\ ab^2\ lt^d/$ $SM5\ al^2\ Cy\ lt^v\ sp^2$	S	:	Star (2-1.3)	Cal. Tech.

Table 1 (continued)

	ab^2	:	abrupt (2-44.0)	
	lt^d	:	light (2-56.)	
8. Tft/SM1,$al^2Cy\ sp^2$	Tft	:	Tuft (2-53.2)	Cal. Tech.
	SM1	:	Balancer for all of chromosome 2	
9. C95-11	wild type		isolated from mutagenized stock 1	U. of A.
10. C92-2			do.	
11. D15-15			do.	
12. D20-4			do.	
13. D22-7			do.	
14. $r^{pyr1\text{-}1}/\hat{XX}/Y$	$r^{pyr1\text{-}1}$	:	pyrimidine requirer (1-54.5)	U. of A.
	$\hat{XX}/Y$	:	C(1) RM, yf/yf (Seattle) backcrossed for ten generations before being used to balance $r^{pyr1\text{-}1}$	
15. $r^{pyr1\text{-}19}/\hat{XX}/Y$	$r^{pyr1\text{-}19}$	:	pyrimidine requirer (1-54.5)	U. of A.
16. $r^{pyr1\text{-}10}/\hat{XX}/Y$	$r^{pyr1\text{-}10}$	:	pyrimidine requirer (1-54.5)	U. of A.
	$\hat{XX}/Y$	:	C(1) RM, $y\ sc\ su(w^a)w^a\ bb/y\ sc^{4L}\ sc^{8R}$ (Cal. Tech.), backcrossed to stock 1 for 6 generations before being used as a balancer for $r^{pyr1\text{-}10}$	

* For further information on mutants and aberrations in stocks 2 - 9 see "Genetic variations of *Drosophila melanogaster* by D. L. Lindsley and E. H. Grell (1968).

Figure 5 . Protocol for an unsuccessful attempt to isolate *Drosophila* auxotrophic mutants on chromosomes 1, 2 and 3, simultaneously. Males in generation 1 were treated with ethyl methanesulphonate and chromosomes marked with an asterisk were derived from these males.

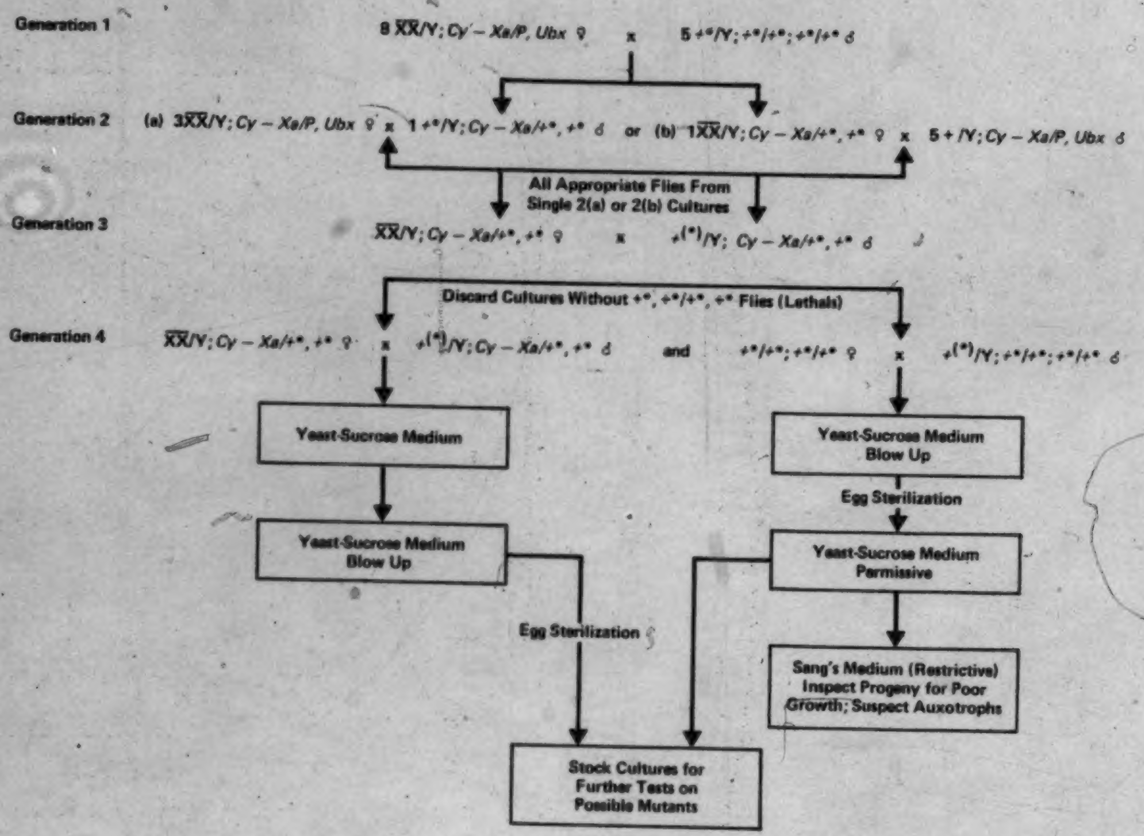
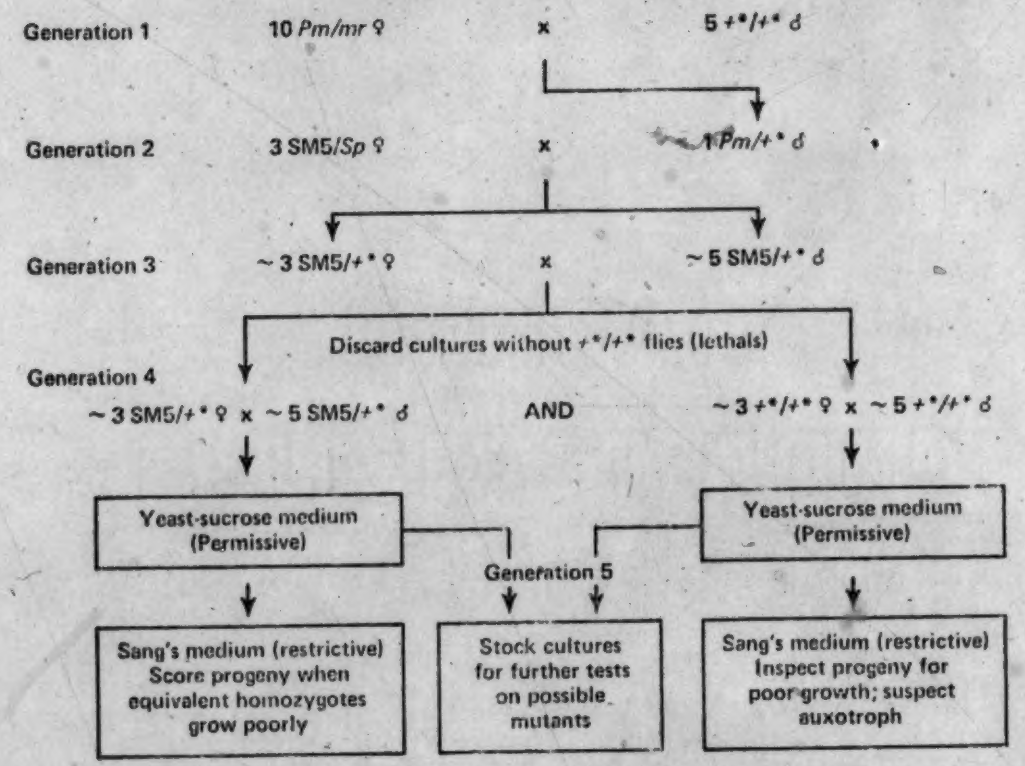

Generation 1
8 $\overline{XX}$/Y; Cy − Xa/P, Ubx ♀ x 5 +*/Y; +*/+*; +*/+* ♂
Generation 2
(a) 3$\overline{XX}$/Y; Cy − Xa/P, Ubx ♀ x 1 +*/Y; Cy − Xa/+*, +* ♂ or (b) 1$\overline{XX}$/Y; Cy − Xa/+*, +* ♀ x 5 + /Y; Cy − Xa/P, Ubx ♂
All Appropriate Flies From Single 2(a) or 2(b) Cultures
Generation 3
$\overline{XX}$/Y; Cy − Xa/+*, +* ♀ x +(*)/Y; Cy − Xa/+*, +* ♂
Discard Cultures Without +*, +*/+*, +* Flies (Lethals)
Generation 4
$\overline{XX}$/Y; Cy − Xa/+*, +* ♀ x +(*)/Y; Cy − Xa/+*, +* ♂ and +*/+*; +*/+* ♀ x +(*)/Y; +*/+*; +*/+* ♂
Yeast-Sucrose Medium
Yeast-Sucrose Medium Blow Up
Egg Sterilization
Yeast-Sucrose Medium Blow Up
Egg Sterilization
Yeast-Sucrose Medium Permissive
Sang's Medium (Restrictive) Inspect Progeny for Poor Growth; Suspect Auxotrophs
Stock Cultures for Further Tests on Possible Mutants

Figure 6 . The protocol used for isolation of second chromosomal auxotrophs. Wild-type males parents (from "Amherst Inbred" line) in generation 1 were treated with ethyl methanesulphonate for 24 hours, using the method of Lewis and Bacher (1968). Chromsomes bearing potential mutants are marked with an asterisk. SM5/*Pm* (referred to as bw^{V1}/SM5 in the text) zygotes produced in the second generation did not survive, facilitating collection of SM5/+* flies.


Generation 1
10 Pm/mr ♀
x
5 +*/+* ♂
Generation 2
3 SM5/Sp ♀
x
1 Pm/+* ♂
Generation 3
~ 3 SM5/+* ♀
x
~ 5 SM5/+* ♂
Discard cultures without +*/+* flies (lethals)
Generation 4
~ 3 SM5/+* ♀ x ~ 5 SM5/+* ♂
AND
~ 3 +*/+* ♀ x ~ 5 +*/+* ♂
Yeast-sucrose medium (Permissive)
Yeast-sucrose medium (Permissive)
Generation 5
Sang's medium (restrictive) Score progeny when equivalent homozygotes grow poorly
Stock cultures for further tests on possible mutants
Sang's medium (restrictive) Inspect progeny for poor growth; suspect auxotroph

allele is involved. A similar mutant (bw^D) had proved the major stumbling block in the previous attempt to substitute the "Amherst" background into the second chromosome balanced stock described above.

Chemicals

The suppliers of compounds used were as follows:

Fisher Scientific Co.: Acetic acid, glacial; Albumin, bovine; Butanol (n-butyl alcohol); Calcium hypochlorite; Cupric sulfate ($CuSO_4 \cdot 5\ H_2O$); Magnesium sulfate (anhydrous); Phenol Reagent solution (Folin and Ciocalteau); Potassium phosphate dibasic (anhydrous); Potassium phosphate monobasic (anhydrous); Propionic acid; Sodium bicarbonate (anhydrous); Sodium carbonate (anhydrous); Sodium citrate; Sodium hydroxide; Sodium phosphate dibasic (anhydrous); Sodium phosphate monobasic (anhydrous); Tris (hydroxy-methyl) amino methane (THAM).

Sigma Chemical Co.: Adenosine; d-Biotin; Calcium pantothenate; Carbamyl-DL-aspartic acid; Carbamyl phosphate; Cytidine; Dihydro-DL-orotic acid; Folic acid; Guanosine; Nicotinic acid (niacin); Orotic acid (anhydrous); Pyridoxine Hcl; Riboflavin; Ribonucleic acid (type V sodium salt); Streptomycin sulfate; Thiamine HCl (aneurin); Uracil; Uridine.

ICN-K and K Laboratories Inc.: Ethyl methanesulfonate (EMS);

ICN-Pharmaceuticals, Inc.: Agar (granulated); Brewer's yeast and Sucrose.

ICN-Nutritional Biochemicals Co.: Casein, vitamin free; Cholestrol (scw); Lecithin (egg).

BDH Chemicals: LB. Oxoid agar No. 3

Calbiochem: Orotidine (cyclohexylammonium salt); 5-Phosphoryl ribose 1-pyrophosphate "PRPP" (dimagnesium salt dihydrate).

Nuclear Research Chemicals Inc.: Orotic acid-6-C14 (5.2 mc/mM) (courtesy of Dr. J. F. Henderson).

J. T. Baker Chemicals Co.: Magnesium chloride and mercapto-acetic acid.

Dispensaries Wholesale Ltd.: Penicillin (1,000,000 I.U.).

Eastman Organic Chemicals: 1 Phenyl-2-Thiourea (courtesy of Dr. R. B. Hodgetts).

Eastman Kodak Co.: Kodak liquid X-ray developer and rapid fixer (courtesy of Dr. K. L. Roy).

All compounds were of the highest standard commercially available whenever impurities could significantly affect normal growth or viability of the flies.

METHODS

Production of media

Media used and their composition are summarized in Table 2.

Twenty-four hours before the preparation of either yeast-sucrose

Table 2: Media used and their composition

Defined Medium

Agar (Oxoid Nº 3)	2.60	g
Casein (Vitamin Free)	5.50	g
Sucrose	750.00	mg
Cholestrol	30.00	mg
Lecithin	400.00	mg
Thiamine	0.2	mg
Riboflavin	0.1	mg
Nicotinic Acid	1.2	mg
Ca Pantothenate	1.6	mg
Pyridoxine	0.25	mg
Biotin	0.016	mg
Folic Acid	0.3	mg
$NaHCO_3$ (anhydrous)	140.00	mg
KH_2PO_4 (anhydrous)	183.00	mg
K_2HPO_4 (anhydrous)	189.00	mg
$MgSO_4$ (anhydrous)	62.00	mg
Streptomycin	20.00	mg
Penicillin *	25,000	iu
Water	To 100	ml

When added:

RNA	400.0 mg
Adenosine	133.6 mg **
Guanosine	141.6 mg **
Inosine	134.1 mg **
Uridine	122.1 mg **
Cytidine	121.6 mg
Adenine	67.6 mg **
Guanine	75.6 mg **
Uracil	56.1 mg
Carbamyl Phosphate	76.5 mg
Carbamyl Aspartate	88.0 mg
Dihydroorotate	79.1 mg
Orotate	78.1 mg
Orotidine	192.7 mg

Dead Yeast-Sucrose Medium

Brewer's Yeast	12.5	g
Sucrose	10.0	g
Granulated Agar	2.0	g

When added:

Penicillin *	25,000 iu
Propionic Acid *	1.0 ml
Streptomycin	20.0 mg
Na_2HPO_4	430.0 mg
NaH_2PO_4	270.0 mg
Water	90 ml

Table 2 (continued)

Egg laying medium

Agar	1.5	g	Propionic Acid *	1.0	ml
Water	100	ml			

Microbial testing medium

Bactoagar	2.0	g	Tryptophan	2.0	mg
Dextrose	2.0	g	Uracil	2.0	mg
Bactopeptone	2.0	g	Histidine	2.0	mg
Bactoyeast extract	1.0	g	Leucine	3.0	mg
Arginine	2.0	mg	Threonine	35.0	mg
Lysine	2.0	mg	Tyrosine	2.0	mg
Adenine	2.0	mg	Phenylalanine	2.0	mg
Methionine	2.0	mg	Water	To 100	ml

* added after autoclaving

** or in appropriate dilution when used at concentrations other than 5.0×10^{-3}M

Modified Drosophila Ringer (EBR's)

H_2O	100.0	ml
Nacl	7.5	g
Kcl	.35	g
$Cacl_2$	.21	g

To make 1 X dilute ten times

medium or Sang's defined medium, test tubes, caps and beakers (in the case of manual pouring) were completely encased in foil and autoclaved for 20 minutes at 121°C on wrapped cycle.

When preparing yeast-sucrose medium, dry ingredients and the appropriate volume of streptomycin were stirred into the appropriate volume of water before autoclaving for 60 minutes at isothermal - 100°C on liquid cycle.

With respect to Sang's medium preparation, all components but agar, casein, sucrose, cholestrol and lecithin were used as stock solutions stored at 4°C. Cholestrol and lecithin were dissolved in 95% alcohol, then the alcohol was gradually evaporated and replaced by double distilled water. When dealing with the preparation of various concentrations of a certain supplement, a stock solution (ten times the highest concentration to be prepared) was made and the various concentrations were prepared by serial dilutions from this stock solution, except in the case of adenosine and guanosine when used at concentrations higher than 5.0×10^{-3}M. It was, then, necessary to weigh the material for each concentration independently.

Microbial testing medium was kindly supplied by Dr. R. C. von Borstel.

Maintenance of axenic conditions

All breeding programs for isolation and characterization of nutritional mutants were carried out under axenic conditions.

Initial sterilization of stocks was made following a modification of the method used by Falk (1973):

Approximately 500 adult flies were left for six hours in a half-pint bottle, coated with a paste of live yeast. The flies were then transferred to a fresh half-pint bottle, tightly closed with a (60 X 15 mm) plastic petri dish containing egg laying medium (Table 2). A scrap of live yeast paste was placed on the side of the dish. The females were allowed to lay eggs for a period of 12 - 15 hours, after which the eggs were collected with a brush and introduced into a screw-capped Kimax culture tube (20 X 150 mm), three-quarters full of freshly prepared filtered 3% calcium hypochlorite. The tube was then filled to the top with calcium hypochlorite, capped and shaken at ten minute intervals for half an hour. Then the cap was removed, the opening of the tube flamed and about three-quarters of the calcium hypochlorite solution discarded. The remaining quarter, containing the eggs, was filtered through sterile filter paper and given two successive rinsings with sterile Drosophila Ringer (Ephrussi and Beadle, 1936). Sterilized embryos were then transferred to a culture tube on a portion of the filter paper.

Established sterile stocks were maintained in shell vials, capped with Kaputs (Bellco Glass Inc.). For routine maintenance, 20 - 30 newly emerged adults were left in tubes for three days then transferred to fresh tubes for one day and discarded. When large numbers of flies from a given stock were required, newly emerged flies were kept in fresh vials for a period of three days, after which they were transferred daily to fresh tubes for about four days. Sterile stocks were grown at 25°C in incubators kept exclusively for axenic cultures.

Handling of flies was carried out either in UV sterilized rooms or enclosed hoods. To minimize the chances of infection, antibiotics were added to the culture medium. Suspect cultures were checked for infection by streaking onto a plastic petri dish (100 X 15mm) containing microbial testing medium (Table 2).

Mutant selection

Compared with systematic isolation of mutations in microorganisms and on the sex chromosome of *Drosophila melanogaster*, which is essentially haploid in males, isolation of autosomal recessive mutations is rather difficult to carry out. This difficulty is increased when axenic cultures are required as is the case with isolating nutritional mutations. The problem can be approached in two basic ways:

The first involves carrying out the mutagenization program under non-axenic conditions, then dealing with the gigantic task of blowing up and sterilizing the mutagenized stocks. This approach places no limitation on the spectrum of balanced lethal systems that could be used to carry out the screen under axenic conditions. The alternative is to carry out the screen under axenic conditions. Although this second approach overcomes most of the shortcomings encountered with the first one, its prerequisite is a mutagenization scheme in which all stages are adequately viable in sterile cultures; additionally, it is impractical to use a dissecting microscope while maintaining axenic conditions, which limits the spectrum of balanced lethal systems that could be used to isolate nutritional mutations to those carrying markers easily detectable with the naked eye. Such systems are fairly rare.

The following section will be concerned with these two aspects of mutant isolation: The protocol in Fig. 5 was initially carried out in an attempt to isolate, under nonaxenic conditions, autosomal conditional nutritional mutations on the first, second and third chromosomes of *D. melanogaster*, simultaneously. However, it soon became evident that the yield of the system is very low (see Table 4) for several reasons. First, the genome of the *Cy-Xa* flies is overloaded with chromosomal aberrations, which resulted in partial inviability of this genotype. Secondly, the scarcity of the *Cy-Xa* genotype, together with excessive handling of the cultures required for the collection of virgin females, culminated in a significant loss of mutagenized stocks owing to severe infection. Thirdly, the presence of numerous translocations and inversions in the balanced lethal system, resulted in several breakdowns of the *Cy-Xa* chromosome, presumably due to interchromosomal effects upon recombination, which made the screen unsuitable. Hence, it was decided to adopt a system that would screen one, rather than three chromosomes, hoping to avoid some of the pitfalls encountered with the previous screen. This screen is shown in Fig. 6. First of all, an attempt at substituting the balancer SM5/bw^{V1} for the $\widehat{XX}$/Y; *Cy-Xa/P, Ubx* one was made. This change would allow screening under axenic conditions, as no microscope is needed to detect the bw^{V1} phenotype. Moreover, the absence of recombinants among some 300 progeny resulting from the cross SM5/ *al dp b pr c px sp* ♀ X *al dp b pr c px sp* ♂, had, already, confirmed Lindsley and Grell's (1968) description of SM5 as the best second chromosome balancer. It turned out, however, that the particular combination SM5/bw^{V1} which was chosen is lethal. This drawback became, nonetheless, the focal point behind the success of the scheme presented in Fig. 6 .

Table 3 : Mutation screen 1 - Success rates in the production of homozygous recessive lethal-free stocks after EMS and control treatment

Treatment	EMS (9.6mM)		Control (1%sucrose)	
Sex of Progeny	♀	♂	♀	♂
Generation 2				
Cultures set	127	47	83	74
Cultures failed *	21	27	4	15
Success	83.5%	42.6%	95.2%	79.7%
Generation 3				
Cultures set	106	20	79	59
Cultures failed *	33	5	21	31
Number of successful tubes	73	15	58	28
Recessive lethals	44	9	2	3
Number of tubes yielding homozygotes	29	6	56	25
Number of tubes yielding homozygotes of only one sex	5	1	-	-
Number of homozygous strains established	24	5	56	25
Success in F_3	22.6%	25%	70.9%	42.4%
Overall success **	18.9%	10.6%	67.5%	33.8%
	16.7%		51.6%	

* Cultures from which it was not possible to set a further generation

** % cultures yielding enough fertile wild-type flies to make homozygous stocks

The detailed operation of this scheme is described in the ensuing section.

Mutagenic treatment and first generation cross

Males 24 - 48 hours old were treated with ethyl-methanesulfonate (EMS) as described by Lewis and Bacher (1968). The concentration of 6.4 mM (0.06 cc EMS in 100 cc 1% sucrose) was used at first, with a yield of 66% recessive lethals as estimated from generation (3) (see RESULTS Table 5). The concentration was then lowered to 4.3 mM (0.06 cc EMS in 150 cc 1% sucrose), giving a yield of 50% recessive lethals. The EMS treatment was carried out in sterilized half-pint milk bottles, containing a pad made of one Kleenex tissue wetted with 10 cc of EMS solution. The bottle was plugged with cotton wrapped in two Kleenex. The bottle and plug were topped with foil to exclude microorganisms. Eighty males were introduced into each bottle. After 24 hours at 25°C they were transferred directly to an empty sterile bottle plugged and topped as described above. To this point the flies were handled in a UV sterilized fume hood rather than in a sterile tissue culture chamber. After one or two hours, the males were etherized and used to set generation (1) cultures, by mating groups of five males to ten virgin bw^{V1}/mr females. The delay in setting the crosses presumably allowed the males to "dry-out" after contact with the EMS solution. Without the delay, they tended to stick to the culture medium after etherization. Prior to mating, the virgins were aged about a week to ensure virginity. Older females also produce progeny at a more precisely predictable time after mating than do younger ones. The parents were normally discarded after seven days.

The second generation cross

Every other day, $bw^{V1}/+^*$ males, from the F_1 progeny were selected and crossed, individually, to three *Sp*/SM5 virgin females. After seven days, the parents were discarded from successful crosses. From the second generation onwards, the origin and fate of each culture was recorded in detail.

The third generation cross

The lethality of SM5/SM5 and bw^{V1}/SM5 genotypes reduced setting the third generation to selection and crossing of SM5/$+^*$ (Cy-winged) sibs or half-sibs. This was easily performed with the naked eye. Imaginal emergence was roughly synchronized by raising the second generation in 25°C incubators with controlled dark-light cycles (12/12 hours), which allowed collection of SM5 progeny at 24 hour intervals, about six hours after "dawn", whilst having the vast majority of the females still virgin. Females mated to non-SM5 males produced either bw^{V1} or excess normal-winged progeny. About five per cent of the cultures were of this kind, so that, since three females were used in each cross, about two per cent of non-virgin females are produced by this method. Individual F_2 progeny were collected until three SM5 females and five SM5 males had been obtained. After five to seven days, depending on the condition of the culture, the parents were discarded.

The third generation progeny were inspected for recessive lethals. To this end, a criterion was set up based on the results of a control run of the mutagenesis screen. In this run, it was found that the F_3 contained a ratio of 0.67: 1 homozygotes to heterozygotes as opposed to the expected ratio of 0.5:1. Using Chi-square and back-calculating from the observed ratio, it was decided to reject an F_3 culture, as containing a recessive lethal, if 25 heterozygotes and no homozygotes were produced. *A priori*, this would lead to rejection of one in ten thousand non-lethal cultures showing the control ratio, a proportion which is obviously irrationally low. However, considering the possibility that auxotrophic phenotypes might exhibit semilethal characteristics, it was considered worthwhile to expend additional effort at this stage.

The fourth generation cross

When the present technique was originally devised, it was intended for the third generation to be the test generation. However, it was found that SM5/+* adults, when raised on Sang's medium, were often difficult to distinguish from +*/+* flies, without careful inspection (see RESULTS). To overcome this shortcoming, it was decided to select for homozygous flies and make of these the test generation. This also presented substantial saving on the cost of Sang's medium, since recessive lethal bearing strains, which normally constituted from 50 - 66% of the third generation, were not tested. In addition, handling the third generation on yeast-sucrose medium helped avoid complications connected with fewer progeny on Sang's medium whilst this was usually overcome once the fourth generation was the test generation. It also turned out that, since maternal genotype is important to the expression of some of the mutants (see RESULTS), the use of the homozygous cultures was essential.

An attempt to use the third generation as the test generation was made. A second chromosome recessive marker would allow for the easy differentiation between SM5/+* and +*/+* adults, when grown on

Sang's medium. Since, as mentioned previously, it was decided to use an intact Amherst second chromosome, induction of such a mutant was necessary. A pupal (2-51.) mutant was soon isolated and used for this purpose. However, its use produced no nutritional mutants probably due to the low viability of *pu/pu* when grown in the same cultures with *pu/*SM5 flies, which made it difficult to discard any culture as being non-nutritional. To overcome the difficulty, it was decided to reject any culture showing at least two pupal flies as non-nutritional. It was not known, then, that heterozygous mothers nutritional mutants could improve the chances of survival for their homozygous progeny (see RESULTS), which presumably made ineffectual the very criterion set for rejecting cultures as non-nutritional.[1]

The fourth generation, as the third, was set up under conditions allowing "virgin" collection, at 24 hour intervals. After five to seven days, the freshly set cultures were transferred from yeast-sucrose to Sang's medium where they remained for three days before returning them to fresh yeast-sucrose medium again for three more days then discarding them.

In cases of sterility of homozygotes, first the type of sterility was determined; then, in the case of male sterility, the cross $+^{*}/+^{*}$ ♀ X $+^{*}$/SM5 ♂ was the test generation; if, on the contrary, the females were sterile, then the reciprocal cross *+*/SM5* ♀ X *+*/+** ♂, was the test generation.

---

[1] A *black* (2-48.5) mutant was isolated and substituted for *pupal*. However, the findings reported above suggested that the test generation should be non-virgin *b/b* ♀ X *b*/SM5 ♂. This would present the advantage of generating internal control segregants, whilst saving virgin collection. Use of the *black* mutant, in this way, has indeed proved successful in the isolation of nutritional mutations (Nash, unpublished data) but is not reported in this study.

Establishment of mutant strains

Prospective mutants were retested three times, using five replicas at a time, before promoting them to the rank of putative auxotrophs. Thereafter, they were kept in two separate lines, one homozygous when possible and the other heterozygous.

Nutritional characterization of mutants

Standard crosses

Three crosses were commonly used to characterize the mutants:

(1) *mutant/mutant* ♀ *X mutant*/SM5 ♂

(2) *mutant/mutant* ♀ *X mutant/mutant* ♂

(3) *mutant*/SM5 ♀ *X mutant/mutant* ♂

The cross:

(4) *mutant*/SM5 ♀ *X mutant*/SM5 ♂

was used initially, but was abandoned because of the maternal effect described in a previous section.

In most experiments crosses (1) and (2) were used. Cross (1) has the advantage that the heterozygous segregants provide an internal control, thus allowing quantitation of the results, as well as testing the adequacy of a particular batch of medium for growth of presumed wild-type flies. Cross (2) gave an absolute measure of mutant productivity on a given medium, providing an external check on the results of cross (1). Cross (2) was also used to monitor the developmental progress of a pure mutant culture which was often useful for devising subsequent experiments. Cross (3) was used mainly in investigation of the maternal effect. Only crosses (1) and (3) could be used in studies on the male sterile strain A66-17.

In all crosses, groups of five males were allowed to mate to three virgin females. In general, adults, or at least adult females, coming from the same producing tube were equally distributed among the different types of media involved in any experiment. Five replicas of each cross were set in most experiments, although this number was sometimes reduced by infections. When dealing with r^{pyr1} mutants (Falk, 1973), the number of female parents, as well as the number of replicas was doubled to overcome the low fertility of these attached-X balanced strains.

Schedule for matings on various media

Adult flies were left to feed on yeast-sucrose for three to five days. When data were obtained from yeast medium, the cultures established in these tubes were scored. The flies were then transferred to Sang's defined media where they remained for three to four days, before transfer to fresh yeast-sucrose tubes for three additional days. The latter set of yeast cultures was mainly used for testing for infection. Variations in the duration of the times allowed for oviposition in the above sequence accommodated scoring previous experiments and availability of freshly prepared Sang's media. These variations are reported in Table 3.

Collection and analysis of data

The results of growth studies were collected at daily intervals by

Table 4: Schedule for matings on various media

Computer Reference for date at which Experiment was set	Title of experiment	Media from which progeny were scored	Number of days on	
			Yeast Medium	Sang's Media
16A4	Initial characterization	Yeast; Sang's + RNA; Sang's	5	3
03B4	Investigation of maternal effects	Yeast; Sang;s + RNA; Sang's	5	3
22D4	Complementation studies	Sang's	4	4
	Localization of mutations			
26H4	Mapping with *S - Sp*	Yeast; Sang's; Yeast (29°C)	5,2*	3
13I4	Mapping with *bw*^{D}	Yeast; Sang's; Yeast (29°C)	5,2*	3
24I4	Mapping with *J*	Yeast; Sang's; Yeast (29°C)	5,2*	3
24J4	Mapping with *Tft*	Yeast; Sang's; Yeast (29°C)	5,2*	4; 3-4
28B4	Determination of ribonucleosides requirement	Sang's; Sang's + AR, +GR, +UR, +CR, +AR+GR+UR+CR	2	4
05E5	Dosage effect of adenosine, guanosine and inosine on *ade2-1*	Sang's; Sang's + various conc. of AR, GR, HR	4	4
25J4	Dosage effect of adenosine and guanosine on *pyr2-1*	Sang's; Sang's + various conc. of AR, GR	4	3
17F4	Dosage effect of adenosine and guanosine on *pyr2-2*	Sang's; Sang's + various conc. of AR, GR	4	4
21K4	Dosage effect of uridine on *pyr2-1* and *-2*	Sang's; Sang's + various conc. of UR	3	4
21C4	Intermediates in *de novo* pyrimidine biosynthesis	Sang's; Sang's + carb. phos.,+ carb. asp., + DHO^-,+ O^-,+ UR	3	4
21E4	Combined effect of orotate and guanosine on *pyr2-1* and *-2*	Sang's; Sang's + O^-, + GR, + O^- + GR	6	4

01H4	Combined effect of orotate and guanosine on *pyr2-1* and *-2*	Sang's; Sang's + O^-, +UR, +O^- + UR	5 - 6	4
21K4	Combined effect of uridine and guanosine on *pyr2-1* and *-2*	Sang's; Sang's + UR, +GR, +UR + GR	3	4
08K4 ; 06C5	Dosage effect of orotidine on *pyr2-1* and *-2*	Sang's; Sang's + various conc. of OR	4	4
09E5	Effect of uracil on *pyr2-1* and *-2*	Sang's; Sang's + U, + UR	4	4
24E4	Detection of temperature sensitivity in *yea* mutants	Yeast (20ºC); Yeast (29ºC)	5;1*;1*	-
10B5; 13E5	Investigation of temperature sensitivity in *yea* mutants	Yeast (29ºC)	5;1*;2*	-
01L5	Dosage effect of adenosine and guanosine on *pyr2-1* and *-2* (con't)	Sang's; Sang's + various conc. of AR, GR	5	3
04L5	Dosage effect of adenine and guanine vs effect of inosine on *pyr2-1* and *-2*	Sang's; Sang's + HR, + various conc. of A, G	0 ***	4

* represents duration of oviposition on other sets of yeast-sucrose tubes that were used in connection with temperature sensitivity

** represents resets on Sang's medium to overcome infertility caused by the interaction of *Tft* with certain mutants

*** parents transferred directly from adenosine to adenine, from guanosine to guanine and from Sang's medium to same or to inosine scoring emerging progeny on the basis of sex and phenotype.

Daily emergence was summed over all cultures grown on a given medium and fed into an IBM/360 Model 67 computer. The APPENDIX shows a printout of the results, expressed as the sum of the number of flies, of a given sex and genotype, produced in a cross; also included is the average productivity per female per day. The APPENDIX also contains the calculated mean time of development, its standard deviation and the median time of development.

Experimental Protocols

Initial characterization

Five putative auxotrophic strains, A66-17, B66-3, B82-4, C2-10 and C42-6, were tested for supplementation by RNA at a concentration of 0.4% using standard crosses 2 and 4. Five other strains: C95-11, C92-2, D15-15, D20-4 and D22-7, picked at random from various mutagenization runs and rejected as nutritional mutants were also used in this test (Table 3: 16A4).

The test showed a significantly higher survival among the homozygous progeny derived from heterozygous parents than among those derived from homozygous parents, when grown on Sang's defined medium. The genetic basis of this phenomenon was investigated using reciprocal crosses between homozygous and heterozygous parents (crosses 1 and 3) (Table 3: 03B4).

Complementation studies

To test for allelism between the putative auxotrophic mutants, homozygous females for each of the five mutants were crossed to homozygous and heterozygous males from each of the five mutants, except for the male sterile strain A66-17 where only heterozygous males could be used (Table 3: 22D4).

Temperature sensitivity

Falk and Nash (1974b) showed that a number of putative auxotrophs are also temperature-sensitive lethals on permissive medium. The five strains were therefore tested for temperature sensitivity: ten replicas of homozygous crosses were set on yeast for five days then transferred to fresh yeast for an additional day before discarding the parents. Half the twenty-four-hour egg-lays were then switched from the usual 25°C temperature to 20°C, the other half to 29°C (Table 3: 24E4).

Two of the five mutants showed heat sensitivity and were retested in a similar manner, using all possible standard crosses (Table 3: 10B5; 13E5).

Genetic mapping

In order to determine the approximate map position of the mutants, all five auxotrophic strains and a control strain (C95-11) were crossed to a series of dominant markers (Table 3: 26H4, 13I4, 24I4, 24J4). The marker chromosomes used were *S*; *Sp*; bw^D; *J* and *Tft*.

Marker/mutant females were backcrossed to homozygous mutant males (except A66-17 where heterozygous males were used), and their progenies on both yeast-sucrose and Sang's media were scored.

Crosses involving *Tft* were of low fertility so that in this case the cross was repeated two or three times.

In strains showing heat sensitivity, the parents were removed from Sang's medium to fresh yeast-sucrose medium where they remained for two days. The latter set of cultures was then switched to 30°C and emerging progenies were also scored.

Recombination frequencies were calculated as follows: For any mutant, m, and dominant marker, D, it was assumed that the phenotypic ratio $D{:}D^+$ (= 1 : s) in the control cross with C95-11 can be taken as a measure of the relative viabilities of D/D^+ and D^+/D^+ and the ratio of SM5/m : m/m (= 1 : y) in the cross SM5/m ♀ X m/m ♂ can be taken as a measure of the relative viabilities of m/m^+ and m/m. Thus if r represents recombination frequency between m and D, the mapping cross $D\,m^+/D^+m$ ♀ X D^+m/D^+m ♂, should yield the following table of genotype frequencies:

Genotype	Frequency
$D\,m^+/D^+m$	1 - r
$D\,m/D^+m$	ry
D^+m^+/D^+m	rs
D^+m/D^+m	(1 - r) sy

Thus, the observed ratio of dominant marker : wild-type in the mapping cross, $D/D^+ : D^+/D^+ = \dfrac{1 - r + ry}{rs + (1 - r)\,sy}$.

This equation might be expected to give fairly reliable estimates of r, providing y is small (that is, for mutants with strong effects under restrictive conditions), given that the relative viability of the dominant mutant estimated in the control cross can be transferred to the mapping crosses. For further discussion of the complexities of this mapping technique see RESULTS.

Supplementation patterns

The mutants that responded to the addition of RNA were tested for supplementation by the four common ribonucleosides, as well as by precursors and potential precursors of pyrimidine and purines. In some experiments two or more of these substances were tested together. The concentrations used ranged from 1 X 10^{-5}M to 5.0 X 10^{-2}M; most commonly, initial tests employed 5 x 10^{-3}M.

Larval transfer

Newly hatched larvae were transplanted to Sang's media at a density of thirty larvae per tube as described by el Kouni and Nash (1974).

Enzymological studies

Orotate phosphoribosyl-transferase was assayed in the following manner.

Preparation of larval extract: Axenic four-day-old adults, at a density of approximately 500/bottle, were allowed to oviposit for 24 hours on egg laying dishes containing defined media. Four days later, the larvae were transferred to a beaker (25 cc.) three-fifths full of ice cold

Drosophila Ringer (EBR). The suspended larvae were stirred vigorously to dissolve any medium picked during the process of larval collection. The larvae were then removed from the suspension by passing it through a sieve and, after rinsing twice, were collected and introduced in tissue grinders (Pyrex, 11 X 80 mm.) containing 0.5 ml ice cold buffer (0.1 M THAM + 0.001 M Phenyl-Thiourea, pH 7.6). After addition of the larvae, the volume of buffer was adjusted to one part by weight of larvae to four parts buffer and ground for 30 seconds at 0°C. A millilitre of ground tissue was then centrifuged (Lourdes Model A-2, 9 RA rotor) in tightly capped one millilitre disposable plastic tubes for ten minutes at 10,000 r.p.m. (12,000 g). The supernatant was transferred into clean one millilitre tubes at 0°C.

Protein determination: It was found that the presence of phenylthiourea in the extract interferes with the method of Lowry *et al* (1951) for protein estimation. To overcome the problem, 100 µl of larval extract were precipitated in 1.0 ml of 10% ice cold solution of trichloroacetic acid. The mixture was then centrifuged (12,000 g for ten minutes), the supernatant removed and the tubes put upside-down to drain. Drained protein precipitates were dissolved in 0.4 ml 0.055 N sodium hydroxide, before using them for protein determination by the Lowry method (op cit.).

Enzyme assay: The conversion, at room temperature, of 6-C^{14}-orotate and PRPP into uridylate, uridine and uracil was measured. The following reaction mixture was prepared:

20 µl	orotate (5 µci/µl)

10 µl	PRPP (20mM)
5 µl	$MgCl_2$ (50 mM)
15 µl	THAM (0.1 M + 1 mMPTU, pH 7.6)
50 µl	larval extract

Fifteen microlitres aliquots were spotted on Whatman 3^{M}_{M} chromatography paper (basic weight 185 g/m^2, thickness 0.33 mm) and chromatographed using a butanol-glacial acetic acid-water solvent (2 : 1 : 1, v/v/v), for approximately eight hours. After drying, the chromatograms were exposed to X-ray films (Kodak NS 54T, no screen, 35.6 X 43.2 cm) for 5 - 7 days. The autoradiograms were then developed and labelled spots were cut out and counted by liquid scintillation in a Searle isocap 200 counter, using a scintillation fluid made of 10.1 g POPOP + 4 g PPO/litre toluene. Specific activity of the enzyme was calculated on the assumption of 80% counting efficiency.

RESULTS

ISOLATION OF MUTANTS

Two types of screen have been used in the course of this study. In the first (Table 5: screen A), the test generation was the fourth generation of the mutagenization screen (Fig. 6). In the second (Table 5: screen B), the test generation was the third generation of the screen shown in Fig. 6.

The overall rate of induced second chromosome nutritional conditional mutations was found to be 0.27%. If screen A were considered alone (since there was no evidence that screen B could produce mutants), this rate would rise to 0.4%. The number of mutagenized chromosomes which gave viable homozygotes was sufficiently low to preclude extensive interpretation of the data. However, the results are comparable with the frequency of production of similar mutants on the X-chromosome where 21/5655 or 0.37% were found (Falk, 1963). The X-chromosome is somewhat smaller than the second chromosome but Falk used higher EMS concentrations.

The lack of mutations in Table 5: screen B has been discussed in MATERIALS AND METHODS.

INITIAL CHARACTERIZATIONS OF NUTRITIONAL CONDITIONAL MUTANTS

Selection for RNA requiring mutants was a main goal of this study. The first step in characterizing the five mutant strains isolated was, therefore, to study their supplementation with RNA. Table 6a and b

Table 5: Results of mutation screen 2

Screen	Mutagenized Stock	EMS conc. in mM	%Rec. lethals	Number of Chrom. Nutritionally tested	Number of Nutritional mutants isolated
A1	*AmOr*$^+$	6.4	65.6	693	1
A2	*AmOr*$^+$	4.3	50.1	545	4
		Screen A:	Total =	1283	Rate = 5/1238
					= 0.004
B1	*pu*	4.3	57.4	66	0
B2	*pu*	8.5	65.7	489	0
		Screen B:	Total =	555	Rate = 0/555
					= 0
		Overall:	Total =	1793	Rate = 5/1793
					= .0027

show the responses of these strains to yeast-sucrose medium, to Sang's defined medium supplemented with RNA and to unsupplemented Sang's medium.

In Tables 6a and 6b results of type 2 crosses (*mutant/mutant* ♀ X *mutant/mutant* ♂) and type 4 crosses (*mutant*/SM5 ♀ X *mutant*/SM5 ♂) are shown. These are repeats of the crosses upon which the initial mutant isolation was based.

Two of the five strains, A66-17 and B66-3, did not respond to RNA. These yeast requirers were not investigated further with respect to dietary characteristics. The other three strains grew satisfactorily on RNA. The ratios of homozygous mutants segregating from heterozygous controls in type 4 crosses, where, *a priori*, 50% is expected, were 37.6% in B82-4, 42.9% in C42-6 and 48.3% in C2-10, when grown on RNA supplemented medium, as opposed to 14.7, 22.0 and 11.3% on unsupplemented medium. Thus, a greater proportion of the mutant progeny from heterozygous parents survived on restrictive medium than would have been predicted from the survival of the progeny of homozygous parents.

In the case of B82-4 and C42-6, the paradoxical behaviour in the different crosses will be shown later to be the result of a maternal effect (Table 6c and d), although some errors in classification cannot be ruled out in this early experiment (see MATERIALS AND METHODS). It is also noticeable that an average delay of three to six days was observed in the development of homozygotes from type 4 crosses compared with the heterozygous segregants, when the crosses were carried out under restrictive conditons. However, this delay disappears with supplementation by RNA. It has been argued by Falk and Nash (1974) that supplemental delay in

Table 6a: Response of various strains to yeast-sucrose medium, Sang's medium supplemented with RNA and Sang's unsupplemented medium.

Type 4 crosses - heterozygous females X heterozygous males

	Number of Progeny		Average Production[a]		Developmental Time Mean ± S.D.		%Rel. Viab.[b]	Developmental Delay (Days)[c]
	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$		
Yeast								
A66-17	345	1355	1.4	5.7	13.0±1.4	11.8±2.5	25.5	1.2
B66-3	243	678	1.6	4.5	12.9±1.4	11.3±1.5	35.8	1.5
C2-10	399	872	2.7	5.8	11.7±1.5	11.3±1.4	45.8	0.4
B82-4	400	818	2.7	5.5	12.2±1.5	11.0±1.6	48.9	1.2
C42-6	372	794	2.5	5.3	11.2±1.4	11.5±1.5	46.9	-0.2
C95-11	389	1316	2.6	8.9	10.4±1.4	10.8±1.4	29.6	-0.4
Sang's RNA								
A66-17	0	1187	0	12.0	-	13.5±2.3	0.0	-
B66-3	4	645	0.07	11.9	17.0±3.6	11.3±1.6	0.6	5.7
C2-10	415	859	5.8	11.9	11.8±1.5	11.6±2.0	48.3	0.2
B82-4	191	508	5.3	14.1	11.8±1.7	11.6±1.8	37.6	0.2
C42-6	331	772	6.1	14.3	11.3±1.8	12.1±2.1	42.9	-0.8
C95-11	128	421	3.6	11.7	11.1±1.7	12.0±2.0	30.4	-1.0
Sang's								
A66-17	0	558	0	5.6	-	14.4±1.6	0.0	-
B66-3	33	511	0.6	9.5	14.6±2.1	15.5±2.2	6.5	-0.9
C2-10	54*	479	1.5	13.3	13.6±1.4	13.9±1.7	11.3	0.3
B82-4	23	156	0.6	4.3	17.7±3.2	14.4±1.8	14.7	3.3
C42-6	61	241	1.4	5.4	19.9±0.9	13.4±1.4	22.0	6.5
C95-11	52	182	1.9	6.7	13.0±2.2	13.0±1.7	28.6	0.0

* Probably misclassification of *m*/SM5 into *m/m* (See text)

[a] Average Production (pfd) : Average number of progeny per female parent per day of oviposition

[b] % Relative viability: (Number of *mutant/mutant (m/m)* divided by number of *mutant*/SM5 (*m*/SM5) progeny) multiplied by 100

[c] Difference between mean development time for *m*/SM5 and *m/m* flies. In general, a delay of one day is statistically significant at the level of 1%. Negative values indicate that *m/m* flies develop faster.

Table 6b: Type 2 crosses - homozygous females X homozygous males

	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Yeast			
B66-3	584	3.9	12.9±1.2
C2-10	1256	8.4	11.4±1.4
B82-4	1413	11.8	11.9±1.7
C42-6	1890	12.6	11.6±1.6
C95-11	2207	14.7	11.9±1.3
Sang's RNA			
B66-3	7	0.13	12.1±1.5
C2-10	790	17.6	12.3±1.5
B82-4	823	18.3	11.8±2.0
C42-6	1321	21.0	11.6±1.9
C95-11	1068	23.7	11.3±1.8
Sang's			
B66-3	10	0.2	14.8±0.9
C2-10	0	0	-
B82-4	15	0.3	22.5±1.3
C42-6	65	1.2	21.1±2.1
C95-11	281	15.6	14.2±1.8

Table 6c: Type 1 crosses - homozygous females X heterozygous males

	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)
	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$		
Yeast								
A66-17	328	891	1.4	3.7	12.8±1.5	12.1±1.5	36.8	0.7
B66-3	301	355	2.0	2.4	12.6±1.2	11.0±1.0	84.8	1.6
C2-10	761	699	5.1	4.7	11.5±1.3	11.0±1.2	108.9	0.5
B82-4	539	567	4.5	4.7	11.3±1.3	11.2±1.3	95.1	0.1
C42-6	692	693	4.6	4.6	11.1±1.2	12.1±0.7	99.9	-1.0
C95-11	523	600	4.4	5.0	10.5±0.9	10.8±1.2	87.2	-0.3
Sang's + RNA								
A66-17	0	23	0	0.2	-	11.5±4.2	0.0	-
B66-3	7	303	0.08	3.4	13.6±1.0	10.8±0.9	2.3	1.4
C2-10	575	581	6.4	6.5	11.6±1.1	11.1±1.0	99.0	0.5
B82-4	520	651	6.4	8.0	11.5±1.1	11.2±1.1	79.9	0.3
C42-6	331	432	3.4	4.8	11.3±1.3	11.0±1.1	76.6	0.3
C95-11	755	797	8.4	8.9	10.7±1.3	11.4±1.6	94.7	-0.7
Sang's								
A66-17	1	108	0.01	0.9	-	14.0±0.9	0.9	-
B66-3	4	187	0.05	2.3	14.3±0.5	12.8±3.3	2.1	1.4
C2-10	7	393	0.08	4.4	17.6±2.1	14.1±2.0	1.8	3.5
B82-4	6	337	0.07	4.2	24.2±3.0	13.2±1.4	1.8	11.0
C42-6	8	341	0.09	3.8	22.8±3.5	14.9±3.3	2.4	7.8
C95-11	423	425	4.7	4.7	12.9±1.6	13.0±1.7	99.5	-0.1

Table 6d: Type 3 crosses - heterozygous females X homozygous males

	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)
	m/*m*	*m*/SM5	*m*/*m*	*m*/SM5	*m*/*m*	*m*/SM5		
Yeast								
B66-3	328	454	2.2	3.0	12.9±1.3	11.2±1.2	72.3	1.7
C2-10	651	655	4.3	4.4	11.2±1.2	11.0±1.2	99.4	0.2
B82-4	572	624	4.2	4.6	11.2±1.3	11.3±1.4	91.7	-0.1
C42-6	637	681	4.3	4.5	10.9±1.2	11.1±1.3	93.5	-0.2
C95-11	391	608	3.3	5.1	10.4±1.1	10.6±1.1	64.3	-0.2
Sang's + RNA								
B66-3	4	748	0.04	8.3	13.8±0.5	11.0±0.9	0.5	2.7
C2-10	790	954	8.8	10.6	11.4±1.1	11.8±1.6	82.8	-0.4
B82-4	630	681	7.0	7.7	11.5±1.1	11.3±1.1	92.5	0.2
C42-6	594	687	6.6	7.6	11.0±1.0	11.0±1.1	86.5	0.0
C95-11	697	873	7.7	9.7	11.0±1.1	11.6±1.4	79.8	-0.6
Sang's								
B66-3	21	493	0.2	5.5	14.9±2.7	12.8±1.8	4.3	2.0
C2-10	22	531	0.2	5.9	22.5±4.0	13.1±1.7	4.1	9.5
B82-4	78	341	1.2	5.4	20.4±2.5	13.8±1.9	22.9	6.6
C42-6	116	307	1.6	4.3	18.2±1.7	13.0±1.6	37.8	5.2
C95-11	368	419	4.5	5.2	12.7±1.3	12.9±1.3	87.8	-0.2

Table 7a: Complementation between the various mutant strains

♂ parent		♀ parent *									
		A66-17		B66-3		C2-10		B82-4		C42-6	
		$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$
$\frac{A66\text{-}17}{SMS}$	Number of progeny	0	30.0	237.0	110.0	165.0	118.0	243.0	97.0	203.0	86.0
	Average production	0	0.5	3.9	1.8	2.8	2.0	4.0	1.6	5.6	2.4
	Mean Development	-	12.7	12.7	12.6	13.3	13.3	12.9	12.7	13.9	13.0
	S.D.	-	1.9	1.4	1.8	1.4	1.8	1.7	1.9	2.0	2.0
$\frac{B66\text{-}3}{SMS}$	Number of progeny	46.0	36.0	2.0	129.0	211.0	112.0	450.0	187.0	**	**
	Average production	0.8	0.6	0.03	2.1	3.5	1.9	7.5	3.1		
	Mean Development	11.9	11.7	13.5	12.2	13.1	13.7	13.8	14.1		
	S.D.	1.6	1.3	2.8	1.6	1.4	1.5	1.9	2.0		
$\frac{B66\text{-}3}{B66\text{-}3}$	Number of progeny	76.0	-	0	-	255.0	-	390.0	-	611.0	-
	Average production	1.3		0		4.3		10.8		10.2	
	Mean Development	11.6		-		12.3		13.8		13.6	
	S.D.	1.1		-		1.5		1.6		1.8	
$\frac{C2\text{-}10}{SMS}$	Number of progeny	47.0	34.0	143.0	53.0	2.0	59.9	197.0	92.0	322.0	102.0
	Average production	0.8	0.6	2.4	0.9	0.04	1.2	4.1	1.9	5.4	1.7
	Mean Development	12.5	12.3	13.1	12.8	31.2	13.6	13.4	13.5	13.1	13.1
	S.D.	2.2	1.1	1.7	1.4	6.2	1.7	1.9	1.8	1.6	1.7
$\frac{C2\text{-}10}{C2\text{-}10}$	Number of progeny	54.0	-	362.0	-	0	-	280.0	-	493.0	-
	Average production	1.1		6.0		0		7.8		8.2	
	Mean Development	11.3		13.1		-		13.7		13.3	
	S.D.	1.8		1.6		-		1.9		1.8	

B82-4 / SM5	Number of progeny	47.0	25.0	230.0	117.0	229.0	116.0	5.0	104.0	26.0	141.0
	Average production	0.8	0.4	3.8	1.9	3.8	1.9	0.1	1.7	0.4	2.3
	Mean Development	11.9	11.6	12.9	12.7	13.2	13.6	20.8	13.2	23.3	12.7
	S.D.	4.2	2.0	1.7	1.9	1.4	1.7	5.4	1.6	3.1	1.8
B82-4 / B82-4	Number of progeny	86.0	-	280.0	-	218.0	-	45.0	-	8.0	-
	Average production	1.4		4.7		3.6		0.7		0.3	
	Mean Development	12.0		12.9		13.2		24.8		23.5	
	S.D.	2.0		1.6		1.5		2.1		2.5	
C42-6 / SM5	Number of progeny	28.0	21.0	184.0	97.0	293.0	168.0	4.0	66.0	14.0	138.0
	Average production	0.5	0.3	3.1	1.6	4.9	2.8	0.1	1.8	0.2	2.3
	Mean Development	12.4	11.6	12.5	12.4	13.3	13.5	21.7	12.9	20.6	12.9
	S.D.	1.6	1.9	1.5	1.5	1.5	1.8	3.4	1.7	3.5	1.7
C42-6 / C42-6	Number of progeny	48.0	-	290.0	-	**	-	44.0	-	37.0	-
	Average production	0.8		4.8				0.7		0.6	
	Mean Development	12.2		12.6				22.8		22.2	
	S.D.	3.2		1.7				2.4		2.6	

* All female parents were homozygous

** Missing data resulted from infected cultures

Table 7b: Complementation between the various mutant strains - Summary

♂ Parents	♀ Parents				
	A66-17	B66-3	C2-10	B82-4	C42-6
A66-17	-	+	+	- +	+
B66-3	+	-	+	+	+
C2-10	+	+	-	+	+
B82-4	+	+	+	-	-
C42-6	+	+	+	-	-

in the development constitutes, by itself, an auxotrophic phenotype.

The other RNA requirer, C2-10 appeared to exhibit a certain leakiness (about 11%) in type 4 crosses grown on unsupplemented medium. It turned out that the majority of the supposed homozygous progeny were the result of a misclassification of *mutant*/SM5 adults as homozygotes. No evident maternal effect could be shown in later tests (Table 6c and d). Moreover, in type 4 crosses grown under restrictive conditions, there was no delay in the development of the "homozygous" flies when compared with the heterozygous segregants. As soon as the possibility of misclassification was recognised, greater care was taken and it then became clear that the rare homozygous survivors do exhibit considerably delayed development.

COMPLEMENTATION BETWEEN MUTANT STRAINS

Homozygous mutant females from all five strains were crossed in all possible combinations to homozygous and heterozygous mutant males, in order to ascertain their complementation patterns. Although it was only strictly necessary to test for allelism between the yeast requirers and between the RNA requirers, it was chosen to investigate the relation between yeast and RNA requirers as an extra measure of control. Use of homozygous females circumvents any possible maternal effects.

The results in Table 7 show complementation in all but 13 of the 43 crosses analyzed. These 13 were either intra-strain crosses or crosses between B82-4 and C42-6. It was therefore concluded that B82-4 and C42-6 are allelic. The two mutant strains showed parallel behaviour in the course of the previous characterization. They both exhibited a certain degree of leakiness accompanied by a pronounced delay in the development of leaky progeny, when grown on Sang's unsupplemented medium. Lethality and developmental delay were substantially suppressed by RNA supplementation. The results also showed that, in general, productivity was correlated with maternal genotype.

It is noticeable that any complementary combination of two mutants had a better survival than the equivalent *mutant*/SM5 segregants. This observation cannot be due to misclassification of adult phenotypes, since the production of *mutant*/SM5 offspring is not conspicuously different in crosses showing the effect and in the crosses in which a genetically similar mother had been used, but the father was taken from the same strain; in such crosses few flies were classified as *mutant/mutant* and it is therefore impossible that a substantial level of misclassification reducing the *mutant*/SM5 class occurred. This observation indicates that the effect must be due to overall improved viability of the complementary combinations. This is supported by the fact that the ratio of homozygous to heterozygous progeny from control strain C95-11, when grown on Sang's defined medium or from the mutant strains when grown on RNA supplemented medium is as expected, thus ruling out any speculation involving inviability specifically associated with the SM5 chromosome.

It is not clear, however, why the heterozygous *mutant*$_1$/*mutant*$_2$ combination should be more viable than the heterozygous *mutant*$_1$ or *mutant*$_2$/SM5 combinations. The effect cannot be simply attributed to hybrid vigour, since the prediction that males should show a lesser effect because they possess a single X-chromosome is not fulfilled.

(See APPENDIX for results).

THE YEAST REQUIRERS

Strain A66-17 (*yea8-1*)

In addition to its requirement for yeast, strain A66-17 was found to be male sterile, with reduced viability in homozygotes (Table 6a and c). Moreover, viability of homozygous females was approximately half that of the homozygous males (Table 8). Homozygous females were also noticeably less productive than the equivalent heterozygotes.

Strain A66-17 was found to be lethal at 29°C (Table 8). However, from 50 - 65% of the progeny of type 1 crosses and from 38 - 43% of type 4 crosses died immediately after emerging, without spreading their wings, so that it is possible that the lethality was imaginal. Heterozygous flies were produced with relatively low frequency at 29°C; particularly, the distortion of sex ratio characteristic of homozygotes at 25°C (less females than males) was found amongst heterozygotes at 29°C. More males than females were found amongst the dead imagos, an observation that can be interpreted in two ways. If the dead flies were mutant homozygotes, this might reflect differential pre-imaginal death amongst the female homozygotes, such as was found at 25°C. However, if they were heterozygotes, the actual severity of sex differential mortality amongst this class at 29°C must, in actuality, be even more extreme than is suggested by the data in Table 8.

The mapping technique described in MATERIALS AND METHODS gives rather unreliable recombination frequencies (r) when mapping semi-

Table 8: Temperature sensitivity of A66-17 (*yea8-1*)

Cross		$\frac{A66\text{-}17}{A66\text{-}17}$ ♀ x $\frac{A66\text{-}17}{SM5}$ ♂				$\frac{A66\text{-}17}{SM5}$ ♀ x $\frac{A66\text{-}17}{SM5}$ ♂			
		Number of Progeny		Average Production		Number of Progeny		Average Production	
Temperatures		$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$
25°C (Five days oviposition)		131	410	0.5	1.7	125	688	0.5	2.9
		197	481	0.8	2.0	220	667	0.9	2.8
	dead imagos	0		0		0		0	
29°C (One day oviposition)*		0	10	0	0.3	0	15	0	1.0
		0	32	0	1.1	0	48	0	3.2
	dead imagos	78		2.6		53		2.5	
29°C (Two days oviposition)*		0	18	0	0.3	0	43	0	1.4
		0	59	0	1.0	0	79	0	2.6
	dead imagos	77		1.3		86		2.9	

* Oviposition was at 25°C

"Dead imagos"died prior to wing expansion lethal mutants. For example, it was found in A66-17 that the survival of homozygotes relative to heterozygous segregants on permissive medium was 56.6%. Attempts to map the viability gene involved showed both negative values and values above the theoretical upper limit of 0.50 for r (Tables 9 and 10). If, nonetheless, the absolute value of r is taken as an indication of linkage, it would then seem that the viability gene maps near *Tft*, the value r = -0.39 being the lowest found, and hence indicating the strongest linkage. Both nutritional requirements and heat sensitivity also seem to map near *Tft* (Table 10). Differences in r between heat sensitivity and nutritional requirement are not significant, (contingency Chi-square tests), so that it is quite likely that all three effects described so far were caused by the same mutation. This observation is important in so far as under such circumstances, the semi-lethality, in permissive conditions, would not affect the mapping of conditional lethality (see discussion of B66-3 below). Neither the gene causing male sterility nor the differential mortality of the two sexes have been mapped. It is to be noted, though, that differential sex mortality is heat sensitive so that it is possible that it is also an expression of the same mutation as heat sensitivity and hence the nutritional requirement and semi-lethality in permissive conditions.

Whether or not the A66-17 mutation is related to *abo* and *da* which map close to the same region and have sex-differential effects (Sandler, 1970, 1972; Parry and Sandler, 1974; Krider and Levine, 1975) and also exhibit temperature sensitivity (Cline, 1975) remains to be seen.

Table 9: Segregation of the control strain C95-11 against five second-chromosome dominant markers

Markers		S			Sp			J			Tft**			bw^D	
Growth Conditions	S	S^+	s*	Sp	Sp^+	s	J	J^+	s	Tft	Tft^+	s	bw^D	bw^{D+}	s
Yeast (25°C) (total emergence)	356	604	1.70	487	473	0.97	315	269	0.85	---	---	---	759	503	0.66
(two days emergence)	166	318	0.52	255	229	0.90	102	71	0.70	---	---	---	234	227	0.97
Yeast (29°C)		***			***		266	292	1.10	---	---	---	441	315	1.05
Sang's (25°C) (total emergence)	450	459	1.02	367	542	1.48	233	429	1.84	---	---	---	415	436	0.71
(five days emergence)	334	197	0.59	192	207	1.08	222	400	1.80	---	---	---	354	328	0.92

		SSp		
	SSp	SSp^+	S^+Sp	S^+Sp^+
Yeast (25°C) (total emergence)	238	118	249	355
(two days emergence)	114	52	141	177
Yeast (29°C)		***		
Sang's (25°C) (total emergence)	249	201	118	341
(five days emergence)	127	75	65	132

* s is the ratio of wild-type segregants : dominant segregants. This value is used in the computation of the recombination frequency (r) of the various mutant effects.

** The cross did not take

*** Inadvertantly discarded

Table 10: Mapping of the semilethality, temperature sensitivity and nutritional requirement of A66-17

Markers	S			Sp			J			Tft			bw^D		
Effects Mapped	S	S^+	r	Sp	Sp^+	r	J	J^+	r	Tft	Tft^+	r	bw^D	bw^{D+}	r
Semilethality	105	140	0.32	161	84	0.04	374	119	-0.15	74	22	-0.39	316	286	0.73
Temperature Sensitivity	77	35	-	78	34	-	169	35	0.16	30	0	0.0	145	91	0.47
Nutritional Requirement	130	163	0.55	191	112	0.28	171	74	0.19	242	21	0.06	147	149	0.49

	SSp			
	SSp	SSp^+	S^+Sp	S^+Sp^+
Semilethality	81	24	80	60
Temperature Sensitivity	60	17	18	17
Nutritional Requirement	113	17	78	85

* $r = \dfrac{\#D^+/D^+ - \#D/D^+}{\#D/D^+\ (s-1) - \#D^+/D^+\ (\frac{s}{y} - 1)}$ as derived from $\dfrac{\#D/D^+}{\#D^+/D^+} = \dfrac{1-r+ry}{rs + (1-r)\ sy}$

where D is the particular second-chromosome dominant marker under consideration

s is the ratio of $wt : D$ (see Table 10)

and y is the ratio of wild-type segregants : SMS segregants ($\frac{\text{A66-17}}{\text{A66-17}} : \frac{\text{A66-17}}{\text{SMS}}$ in this case)

Strain B66-3 (*yea9-1*)

Besides its requirement for yeast, strain B66-3 was characterized by a reduction in viability on permissive medium (Table 6a, c, and d). Moreover, viability seemed to be maternally influenced. The relative viability of homozygotes, based upon an expectation of normal Mendelian segregations, was 85% when the mother was homozygous (type 1 crosses) and 72% when the mother was heterozygous (types 3 and 4 crosses). However, homozygous mothers are less productive than the heterozygous ones (Table 6a,c and d) so that the observed maternal effect on viability may, in fact, be a result of greater competition between homozygotes and heterozygotes in more crowded cultures.

Another characteristic of strain B66-3 when grown on permissive medium, was an average delay of one and a half days in the development of homozygotes compared with the heterozygous segregants (Table 6a, c, and d). Strain B66-3 was also found to be a heat sensitive lethal mutant (Table 11). In contrast to A66-17, B66-3 heterozygotes seem to grow normally at 29°C.

The viability factor in B66-3 seems to map near *Sp* (Table 12). It is true that r for *S* is negative, however, the *S* class of progeny is very low in all six different strains involved in the mapping experiments due to a severe reduction in *S*, Sp^+ progeny grown on yeast-sucrose medium (the *S* and *Sp* data were all obtained from crosses to *S*, *Sp*). Therefore, it was assumed that this abnormality may be due to some interaction between *S* and the right portion of the "Amherst" second chromosome. Inspection of the data for the first two days of emergence, shows the

Table 11: Temperature sensitivity of B66-3 (*yea9-1*)

Cross	$\frac{\text{B66-3}}{\text{B66-3}}$ ♀ x $\frac{\text{B66-3}}{\text{SM5}}$ ♂				$\frac{\text{B66-3}}{\text{SM5}}$ ♀ x $\frac{\text{B66-3}}{\text{B66-3}}$ ♂				$\frac{\text{B66-3}}{\text{B66-3}}$ ♀ x $\frac{\text{B66-3}}{\text{B66-3}}$ ♂	
	Number of Progeny		Average Production**		Number of Progeny		Average Production**		Number of Progeny	Average Production
Temperature	$\frac{m}{m}$	$\frac{m}{\text{SM5}}$	$\frac{m}{m}$	$\frac{m}{\text{SM5}}$	$\frac{m}{m}$	$\frac{m}{\text{SM5}}$	$\frac{m}{m}$	$\frac{m}{\text{SM5}}$		
25°C (Five days oviposition)	159	180	1.1	1.2	164	245	1.1	1.6	287	1.9
	142	175	1.0	1.2	164	209	1.1	1.4	296	2.0
29°C (One day oviposition)*	0	56	0	3.7	0	66	0	4.4	0	0
	0	67	0	4.5	0	52	0	3.5	0	0
29°C (Two days oviposition)*	0	112	0	3.7	0	105	0	3.5	0	0
	0	83	0	2.8	0	114	0	3.8	0	0

* Oviposition was at 25°C

** The difference in productivity is probably accounted for by the fact that flies grown at 25°C originated from less mature and initially unmated parents factor responsible for the delay in development to map near *Sp* (Table 12) so that both reduced viability and developmental delay may be caused by the same genetic determinant. Heat sensitivity and nutritional requirement, on the other hand, seem to map around *Tft* (Table 12). Since the difference between them is statistically non-significant, they probably map at the same position. It is to be noted that, whenever a semilethal is associated with a conditional lethal, but maps at some other location, excess dominant segregants are expected, except in the case where the viability factor is located between the dominant marker and the conditional mutation. Thus, the nutritional requirement would appear to be linked more strongly to the dominant than is, in fact, the case. Thus when several dominants are used, the nutritional requirement may seem to be linked to a number of them. In the case of B66-3, this expectation is clearly fulfilled.

In both yeast requirers, the nutritional requirement and heat sensitivity appear to be two aspects of the same mutation. A similar relationship has been reported by Falk (1973). It was argued by Falk that since EMS-derived, sex-linked, temperature-sensitive mutations are 6.2% as common as sex-linked recessive lethal mutations and since, contrary to the expectations based on these results, all yeast-requiring mutants turn out to be temperature sensitive, the possibility arises that temperature sensitivity of yeast requirers may have a different basis than thermolability (Suzuki, 1970 and Camfield and Suzuki, 1972). As an example, Falk suggests that growing organisms at high temperatures may increase their demands for particular metabolites, so that if these metabolites were just sufficient to allow growth under permissive con-

Table 12: Mapping semilethality, developmental delay, temperature sensitivity and nutritional requirement of B66-3 (*yea9-1*)

Markers		S			Sp			J			Tft			bw^D	
Effects Mapped	S	S^+	r	Sp	Sp^+	r	J	J^+	r	Tft	Tft^+	r	bw^D	bw^{D+}	r
Semilethality	440	485	-0.14	538	387	0.04	451	417	0.63	110	114	0.45	609	631	1.18
Developmental Delay	179	161	1.68	273	67	-1.27	140	64	0.68	11	4	-	261	222	0.42
Temperature Sensitivity	237	178	-	323	92	-	332	25	0.06	57	0	0.0	527	220	0.37
Nutritional Requirement	459	344	0.42	492	311	0.28	378	199	0.20	212	52	0.13	312	240	0.42

		SSp		
	SSp	SSp^+	S^+Sp	S^+Sp^+
Semilethality	338	102	200	287
Developmental Delay	173	6	100	61
Temperature Sensitivity	217	20	106	72
Nutritional Requirement	347	112	145	199

ditions, increasing the need for them, by raising the temperature of growth, would simply result in lethality.

Since the newly isolated yeast requirers are also found to be temperature sensitive, with the temperature sensitivity once again mapping at the same location as the nutritional requirement, it may well be that the relationship between these two effects is indeed highly specific and, perhaps, has a more definitive root cause than was suggested by Falk. Since membranes are semi-fluid structures that could possibly modify their phases in response to temperature changes, it would appear that membranes are likely candidates for causing temperature sensitive lethality. Therefore, it is possible that yeast-requiring mutants may be deficient in metabolite(s) required in general metabolism as well as membrane formation. The requirement can be met by feeding on yeast-sucrose at 25°C. However, this does not mean that the deficient metabolite(s) is totally substituted by exogenous yeast so that at altered temperatures the membrane might not respond normally. Fatty acids, steroids, and carbohydrates are all constituents of some membrane systems, all are basically derived from diet and all are metabolically modified to some extent. Mutants disrupting their metabolism might well yield the double phenotype associated with all yeast-requiring strains so far described.

Falk has discussed various approaches to the solution of the yeast requirement problem. Additional approaches would be the investigation of the responses to specific inhibitors in cultured cells of the yeast-requiring mutants. For example, their response to polyenes may lead to a clue as to whether or not the yeast requirers are deficient in cholestrol metabolism (Molzahn and Woods, 1972).

Yeast-requiring mutants could also be fed with yeast-sucrose medium in which the yeast is replaced by yeast from cells deficient in metabolisms of vital metabolites, e.g. as *ole 1* and *erg 1* (Plischke *et al.*, 1975). Development of the flies on various deficient cells could be used to monitor the specific requirement of the tested flies.

THE RNA REQUIRERS

Determination of ribonucleoside requirements

Mutant strains that responded to the addition of RNA to restrictive medium were tested for their ribonucleoside requirements. The dose of 5 X 10^{-3}M was used since it corresponds approximately to the optimum RNA concentration for wild-type flies (Sang, 1956).

Strain C2-10 was found to require adenosine (AR) (Table 13a). Most of the flies which survived in early experiments on both guanosine (GR) and uridine (UR) and were classified as homozygous mutants are likely to have been misclassifications since they were more common and had exhibited far less developmental delay than those found in subsequent experiments.

Strain B82-4 exhibited a complex response to the various ribonucleosides. In crosses where the female parents were homozygous whilst the male were heterozygous (type 1 crosses), it apparently responded well to both pyrimidine ribonucleosides and to guanosine. Relative viabilities of mutant offspring were 108.2% on uridine, 88.3% on cytidine (CR) and 152.5% on guanosine. The response to adenosine seemed

Table 13a; Response of the RNA requirer C2-10 (*ade2-1*) to various ribonucleosides

$\frac{C2\text{-}10}{C2\text{-}10}$ ♀ x $\frac{C2\text{-}10}{SM5}$ ♂

Supplement (5.0×10^{-3}M)	Number of Progeny $\frac{m}{m}$	Number of Progeny $\frac{m}{SM5}$	Average Production $\frac{m}{m}$	Average Production $\frac{m}{SM5}$	Developmental Time Mean ± S.D. $\frac{m}{m}$	Developmental Time Mean ± S.D. $\frac{m}{SM5}$	%Rel. Viab.	Developmental Delay (Days)
None	0	332	0	5.5	-	14.1±1.8	0.0	-
AR	74	42	1.2	0.7	12.9±1.9	12.4±1.5	176.2	0.5
GR	7*	80	0.2	1.3	12.6±3.1	14.1±1.9	8.8	-1.5
UR	5*	346	0.08	5.8	16.4±1.1	14.9±1.8	1.5	1.5
CR	0	288	0	6.0	-	15.1±1.9	0.0	-
AR+GR+UR+CR	282	421	4.7	7.0	12.2±1.9	12.6±2.2	67.0	-0.4

$\frac{C2\text{-}10}{SM5}$ ♀ x $\frac{C2\text{-}10}{C2\text{-}10}$ ♂

Supplement (5.0×10^{-3}M)	Number of Progeny $\frac{m}{m}$	Number of Progeny $\frac{m}{SM5}$	Average Production $\frac{m}{m}$	Average Production $\frac{m}{SM5}$	Developmental Time Mean ± S.D. $\frac{m}{m}$	Developmental Time Mean ± S.D. $\frac{m}{SM5}$	%Rel. Viab.	Developmental Delay (Days)
None	4*	706	0.07	11.8	15.0±1.3	14.6±1.8	0.6	0.4
AR	81	88	1.4	1.5	12.8±1.5	12.4±1.4	92.1	0.4
GR	25*	85	0.5	1.8	18.1±2.4	14.2±2.1	29.4	3.9
UR	7*	559	0.1	9.3	19.6±3.5	14.9±1.7	1.3	4.7
CR	9*	430	0.2	7.2	18.0±3.4	14.1±1.9	2.1	3.9
AR+GR+UR+CR	337	441	4.0	7.4	12.2±2.0	13.4±2.5	75.4	-1.2

* Probably misclassified $\frac{C2\text{-}10}{SM5}$ flies

Table 13b: Response of the RNA requirer B82-4 (*pyr2-1*) to various ribonucleosides

Supplement (5.0 X 10^{-3}M)	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)
	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$		
	$\frac{B82\text{-}4}{B82\text{-}4}$ ♀ X $\frac{B82\text{-}4}{SM5}$ ♂							
None	8	423	0.1	7.1	20.5±5.6	13.9±1.7	1.9	6.6
AR	14	75	0.2	1.3	15.9±1.5	12.7±1.7	18.7	3.2
GR	154	101	2.6	1.7	18.8±2.5	14.5±2.1	152.5	4.3
UR	397	367	6.6	6.1	16.4±2.3	15.0±2.7	108.2	1.4
CR	369	418	6.2	7.0	16.6±2.1	14.9±2.3	88.3	1.7
AR+GR+UR+CR	258	367	4.3	6.1	11.8±1.8	12.2±2.1	70.3	-0.4
	$\frac{B82\text{-}4}{SM5}$ ♀ X $\frac{B82\text{-}4}{B82\text{-}4}$ ♂							
None	66	436	1.1	7.3	19.8±2.3	14.0±1.6	15.1	5.8
AR	75	179	1.3	3.0	14.1±1.5	13.2±1.6	41.9	0.9
GR	75	185	1.3	3.1	17.6±2.2	14.4±1.4	40.5	3.1
UR	387	467	6.4	7.9	17.0±2.2	15.6±2.4	82.9	1.4
CR	312	372	5.2	6.2	16.6±2.1	15.8±2.2	83.9	0.8
AR+GR+UR+CR	278	456	4.6	7.6	12.1±1.8	12.1±1.9	61.0	-0.0

Table 13c: Response of the RNA requirer C42-6 (*pyr2-2*) to various ribonucleosides

	$\frac{C42\text{-}6}{C42\text{-}6}$ ♀ x $\frac{C42\text{-}6}{SM5}$ ♂							
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)
Supplement (5.0 X 10^{-3}M)	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$		
None	7	342	0.1	5.7	19.0±4.7	13.7±1.5	2.1	5.3
AR	13	63	0.2	1.1	14.9±2.9	12.2±1.5	20.6	2.7
GR	76	165	1.3	2.8	18.9±2.4	14.5±1.8	46.1	4.4
UR	405	400	6.8	6.7	16.3±2.2	15.1±2.6	101.3	1.2
CR	399	356	6.7	5.9	16.2±2.2	14.7±2.1	112.1	1.5
AR+GR+UR+CR	361	447	6.0	7.5	12.3±2.0	12.8±2.3	80.8	-0.5
	$\frac{C42\text{-}6}{SM5}$ ♀ x $\frac{C42\text{-}6}{C42\text{-}6}$ ♂							
None	115	480	1.9	8.0	19.5±2.2	14.6±1.9	24.0	4.9
AR	73	180	1.2	3.0	13.4±1.6	12.8±1.6	40.6	0.6
GR	131	211	2.2	3.5	16.8±2.5	14.5±2.1	62.1	2.3
UR	354	398	5.9	6.6	16.2±1.9	15.7±2.2	89.0	0.5
CR	319	380	5.3	6.3	17.0±2.2	16.5±2.6	84.0	0.6
AR+GR+UR+CR	364	387	6.1	6.5	12.5±2.0	14.4±2.5	94.0	1.9

less adequate. The relative viability of homozygotes was only 18.7%. Considering this criterion alone, guanosine gave the best response. However, the internal controls are incongruous in the purine nucleoside tests; whilst the average production of *mutant*/SM5 offspring on restrictive medium was 7.1 per female per day (pfd), on guanosine this figure dropped to 1.7 pfd and on adenosine it was 1.3 pfd. Larval transfer experiments with both C95-11 and "Amherst" control strains (el Kouni, unpublished) show adenosine and guanosine to be toxic, so that this reduction in productivity probably represents sensitivity of developing flies to the two purine ribonucleosides. It would thus appear that the number of flies produced on either guanosine or adenosine supplemented media is a resultant of the interplay between supplementation by a ribonucleoside and resistance to its toxicity. Since the contribution of each of these two components is not known, results may be misleading. The most anomalous result, for example, is the excessive relative survival (152.5%) of B82-4 homozygotes derived from type 1 crosses on guanosine. Clearly, survival of relatively more homozygotes cannot represent creation of new mutant flies by guanosine. The response to guanosine must then be a reflection of higher guanosine sensitivity amongst *mutant*/SM5 flies than amongst *mutant/mutant* flies. This not only casts doubts on the conclusion that guanosine is a better supplement than the pyrimidine nucleosides, but also leaves open the possibility that guanosine may turn out to be quite inadequate as a supplement. It is paradoxical that this result is specific to the particular cross and mutant; the relative viability of homozygotes from the reciprocal cross involving heterozygous mothers and homozygous fathers (type 3 crosses) was only 40.5% on guanosine. Relative viability of homozygotes from type 3 crosses on both uridine and cytidine were, on the other hand, only a little lower (82.9 vs 108.2% on UR and 83.9 vs 88.3% on CR). These small differences in viability are probably trivial; they might, for example, be accounted for by greater competition between homozygous and heterozygous segregants in slightly more crowded cultures produced by heterozygous mothers.

Strain C42-6, shown by complementation to be an allele of B82-4, exhibited a behaviour parallel to that of B82-4 except for the response of progenies from type 1 crosses to guanosine. In this case, relative viability of homozygotes was only 46.1% in contrast to the 152.2% figure obtained with B82-4. This fact casts further doubts concerning the interpretation of supplementation by guanosine since a reduction in the sensitivity expressed as an increase in the number of C42-6/SM5 survivors (2.8 pfd) relative to the B82-4/SM5 survivors (1.7 pfd) is a major factor in lowering the apparent "response" to guanosine to less than one third its value with B82-4 (46.1/152.5 = 0.30).

It is concluded that among the three RNA requirers, C2-10 is an adenosine requirer, whilst B82-4 and C42-6 respond to some extent to all four ribonucleosides, with a suggestion that their best response is to pyrimidine ribonucleosides.

The adenosine requirer, C2-10 (*ade8-1*)

In contrast to the other mutants reported in this study, strain C2-10 was both of high viability on the permissive yeast-sucrose medium and was singularly inviable on the restrictive medium. It is, therefore, expected that its mapping would not show any of the complications encountered with the other mutants (Table 14). Applying the two-point cross method that has been used in this study so far, resulted in the values $r = 0.04$ between the mutant and *Sp* and $r = 0.28$ between the mutant and *S*, which places the genetic determinant for the nutritional requirement of C2-10 just to the right of *Sp*. However, analysis of a three-point cross involving the dominant markers *S* and *Sp* and adjusting the figure with respect to the information from Lindsley and Grell (1968), showed the nutritional requirement of C2-10 to be located at some 5.0 map units to the left of *Sp* (Table 14).

It should be noted, however, that the penetrance of both *Sp*, *J* and *Cy* is reported to be less than 100%, especially at temperatures lower than 25°C (Lindsley and Grell, 1968). *Cy* also overlaps wild-type when grown on Sang's defined medium. Thus, it is possible that both *Sp* and *J* also exhibit such an overlap on Sang's medium. Indeed, the results from strain C95-11 with *Sp* and *J* on restrictive medium showed excess wild-type progeny relative to either *Sp* or *J*. Under such circumstances, penetrance of *Sp* and *J* may influence the recombination values calculated with these markers, so that the nutritional requirement of C2-10 may be nearer to *Sp* than actually found.

The data also show the nutritional requirement of C2-10 to be linked to *Tft* (Table 14). This raises the possibility that C2-10 is a double mutant. However, this would imply that all wild-type segregants in the test with *J* should be double recombinants. Therefore, there should be very few relative to the *J* segregants, which is quite incompatible with the observed results, although the possibility that *J* overlaps wild-type weakens this conclusion. It is also possible that the

Table 14: Mapping the nutritional requirement of C2-10 (*ade2-1*)

(a) Single dominant markers

Marker(s)	Segregants	*D*	D^+	*r*
S	Number of segregants	438 (474)*	182 (727)	0.28
Sp	Number of segregants	564 (636)	56 (565)	0.04
J	Number of segregants	131 (371)	137 (597)	0.36
Tft	Number of segregants	119 (193)	39 (178)	0.19
bw^D	Number of segregants	277 (667)	291 (740)	0.50

(b) Three point cross with *S Sp*

		SSp^+	S^+Sp	S^+Sp^+	SSp
SSp	Number of segregants	50 (172)	176 (334)	6 (393)	388 (302)
	r**	5.18 (*Sp-m*)		16.82 (*S-m*)	

* Number in parenthesis represents equivalent data on yeast sucrose medium

** These values of r were calculated by an entirely different method from those shown above, which tend to indicate that the mutant is outside the *S* - *Sp* interval. However, if this were the case the double recombinant class in the three-point cross would be SSp^+ whereas it appears to be S^+Sp^+. Thus, assuming the mutant to be within the *S* - *Sp* region, the published map distance, 22.0 (Lindsley and Grell, 1968) has been partitioned in the ratio of presumed recombinants between *S* and mutant (176+6=182) to the presumed recombinants between *Sp* and the mutant (50+6=56).

discrepancy associated with the *Tft* : + segregation is the result of an interaction in which the combination of the left portion of *Tft* and the right portion of C2-10 chromosomes is semi-lethal on restrictive medium. This is similar to the previously suggested semi-lethality of the combination between the left portion of *SSp* and the right portion of "Amherst" chromosome on permissive medium. It is equally possible that the nutritional requirement of C2-10 maps around *Tft* whilst the linkage with *Sp* could be due to the creation of a synthetic lethal arising from the combination right portion of "Amherst" and left portion of *SSp*. The strongest evidence suggests the existence of a locus close to *Sp*, which yields nutritional conditional mutants with lowered viability on restrictive medium and restored viability on adenosine supplemented medium. This locus has been designated *ade2* and strain C2-10 carries the mutant allele *ade2-1*.

The dose of 5 X 10^{-3}M, used to define the ribonucleoside requirements of *ade2-1* need not be optimal. This dose represents a conversion from the optimal concentration of RNA for stimulation of development rate in wild-type flies (Sang,1956). In this study it has been found that supplementation with purines was accompanied by a strong reduction in the number of *mutant*/SM5 adults produced. Therefore, the dose response of *ade2-1* to adenosine and guanosine as well as to inosine (HR) was investigated. The results are summarized in Table 15.

The mutant seems to respond to both adenosine and inosine. However, no significant response to guanosine was observed at any of the concentrations tested. The concentrations of 10^{-2}M was optimal for both adenosine and inosine; both adult production and developmental rate were at

Table 15a: Dose response of *ade2-1* (C2-10) to adenosine

Cross	$\frac{ade2\text{-}1}{ade2\text{-}1}$ ♀ X $\frac{ade2\text{-}1}{SM5}$ ♂								$\frac{ade2\text{-}1}{ade2\text{-}1}$ ♀ X $\frac{ade2\text{-}1}{ade2\text{-}1}$ ♂		
	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	1	152	0.02	2.5	-	13.9±1.9	0.7	-	9	0.2	27.0±1.6
1.0 X 10^{-3}M	5	243	0.08	4.1	17.3±3.3	13.0±1.6	2.1	4.3	16	0.3	19.1±2.3
3.2 X 10^{-3}M	153	187	2.6	3.1	13.2±1.7	13.1±1.5	81.8	0.1	329	5.5	13.0±1.7
1.0 X 10^{-2}M	299	233	5.0	3.9	12.8±1.5	12.7±1.4	128.3	0.1	596	9.9	12.8±1.1
3.2 X 10^{-2}M	21	101	0.4	1.7	16.4±2.4	15.0±2.0	20.8	1.4	48	0.8	16.1±1.3

Table 15b: Dose response of *ade2-1* (C2-10) to guanosine

Cross	$\frac{ade2\text{-}1}{ade2\text{-}1}$ ♀ x $\frac{ade2\text{-}1}{SM5}$ ♂								$\frac{ade2\text{-}1}{ade2\text{-}1}$ ♀ x $\frac{ade2\text{-}1}{ade2\text{-}1}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	1	152	0.02	2.5	-	13.9±1.9	0.7	-	9	0.2	27.0±1.6
1.0 X 10^{-3}M	1	179	0.02	3.0	-	14.0±2.3	0.0	-	0	0.0	-
3.2 X 10^{-3}M	4	123	0.07	2.1	26.3±1.7	14.4±1.9	3.3	11.8	1	0.02	-
1.0 X 10^{-2}M	4	333	0.07	5.6	24.5±8.2	15.9±2.6	1.2	8.6	0	0.0	-
3.2 X 10^{-2}M	3	122	0.05	2.0	25.2±4.0	17.4±4.1	2.5	7.8	0	0.0	-

Table 15c: Dose response of *ade2-1* (C2-10) to inosine

Cross	$\frac{ade2\text{-}1}{ade2\text{-}1}$ ♀ x $\frac{ade2\text{-}1}{SMS}$ ♂								$\frac{ade2\text{-}1}{ade2\text{-}1}$ ♀ x $\frac{ade2\text{-}1}{ade2\text{-}1}$ ♂		
	Number of Progeny		Average Pro- duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro- duction	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$					
None	1	152	0.02	2.5	-	13.9±1.9	0.7	-	9	0.2	27.0±1.6
1.0 X 10^{-3}M	-	-	-	-	-	-	-	-	20	0.3	17.6±2.3
3.2 X 10^{-3}M	254	166	5.3	3.5	12.6±1.4	12.5±1.5	153.0	0.1	421	7.0	12.6±1.4
1.0 X 10^{-2}M	670	642	7.0	6.7	13.0±1.3	13.0±1.4	104.4	-0.0	-	-	-
3.2 X 10^{-2}M	30	106	0.5	1.8	16.2±1.3	14.4±1.3	28.3	1.8	-	-	-

their best at this dose. At lower and higher doses, both drop off considerably. It seems, however, that the effect of the higher dose is a result of toxicity rather than lack of supplementation since controls show a similar effect.

In general, female productivity on inosine was higher than on adenosine, with a two-fold difference at the optimum concentration. This could be accounted for by differences in egg production, egg hatchability and postembryonic mortality. It is to be noted that egg production and egg hatchability represent the effects of the medium on the mothers whereas postembryonic mortality is presumably the response of offspring to the medium. In order to distinguish between these two elements, postembryonic mortality was measured. For this purpose, first instar homozygous *ade2-1* and C95-11 larvae were transferred from non-nutrient medium to inosine, adenosine and guanosine supplemented media as well as to unsupplemented medium, using the technique described by el Kouni and Nash (1974). Results (Table 16) with inosine showed the larval and pupal stages to survive best at a concentration of 10^{-2}M and appreciable survival was observed at 3.2 X 10^{-3}M, quite in agreement with the results obtained when females were allowed to oviposit on the nutrient media. With adenosine, however, the best survival was at the concentration of 3.2 X 10^{-3}M whilst at a concentration of 10^{-2}M, the survival dropped to half its value for the former dose. This is in contradiction to the results obtained in the earlier experiments where the best productivity was found at 10^{-2}M adenosine. One should remember, though, that the survival of mutants is a resultant of supplementation by and sensitivity to purine ribonucleosides. Therefore, the discrepancy be-

Table 16: Growth of transplanted $\frac{ade2\text{-}1}{ade2\text{-}1}$ larvae on purine ribosides

Supplement	Concentration (Molar)	Number of larvae transferred	Number of pupae formed	Number of adults emerged	% Survival
None		270 (120)	1 (80)	1 (75)	1.6 (62.5)
HR	1.0×10^{-3}	120 (120)	-- (34)	17 (31)	22.7 (41.3)
	3.2×10^{-3}	300 (120)	112 (47)	79 (34)	42.1 (45.3)
	1.0×10^{-2}	210 (240)	75 (30)	61 (20)	46.5 (13.3)
	3.2×10^{-2}	210 (240)	30 (12)	18 (8)	13.7 (5.3)
AR	1.0×10^{-4}	180	1	0	0.0
	3.2×10^{-4}	180	10	0	0.0
	1.0×10^{-3}	300 (120)	110 (25)	58 (20)	30.9 (26.7)
	3.2×10^{-3}	330 (180)	111 (56)	85 (40)	41.2 (35.6)
	1.0×10^{-2}	300 (150)	69 (14)	35 (11)	18.7 (11.7)
	3.2×10^{-2}	90 (120)	10 (10)	5 (6)	8.9 (8)
GR	1.0×10^{-4}	180	2	0	0.0
	3.2×10^{-4}	180	11	0	0.0
	1.0×10^{-3}	270 (120)	8 (39)	7 (37)	4.2 (49.3)
	3.2×10^{-3}	300 (120)	0 (27)	0 (23)	0.0 (30.7)
	1.0×10^{-2}	300 (120)	0 (48)	0 (28)	0.0 (37.3)
	3.2×10^{-2}	120 (120)	0 (45)	0 (24)	0.0 (32.0)

Numbers in parenthesis represent equivalent data for the control C95-11 tween the two results might be accounted for by a slight shift in the balance between these two elements, created by differences in culture conditions that were generated in the two kinds of experiments. For example, in the case when mothers oviposit on supplemented Sang's medium, large numbers of larvae are present and, in type 1 crosses, heterozygous and homozygous mutants coexist. This may expose the supplement, so that it becomes accessible to the mutants. In the case of larval transfer experiments, only 30 homozygous larvae are introduced to each tube so that the supplement may not be as readily available. When supplementing with adenosine, the toxicity of the compound results in the survival of fewer larvae, which in turn results in a minimal exposure of the supplement so that an additional cause of mortality arises. Supplementing with the less toxic inosine allows more survivors and results in the exposure of more supplement, so that most of the observed mortality is probably due to toxic effect of inosine alone. An analogous example of a probable effect of culture conditions is found in the observation that escapees (homozygous survivors on unsupplemented medium) produced by homozygous mothers develop at different rates depending on the paternal genotype. When homozygous fathers are used, the cultures contain very few viable progeny and these progeny develop slowly (27 days). On the other hand, when heterozygous fathers are used, the development period is reduced to 20 days. The latter cultures are, of course, relatively crowded due to the presence of heterozygous segregants.

Despite the fact that there is no absolute correspondence between larval transfer experiments and oviposition experiments it may nontheless be concluded that post embryonic mortality can account for a great deal of the variation in female productivity on the various media, since, under optimum conditions, more than 50% of the implanted *ade2-1/ade2-1* larvae never reach adulthood (Table 16).

ade2-1 exhibited a certain resistance to the toxicity of adenosine and inosine relative to the internal controls, *ade2-1*/SM5. For example, at the concentration of 1.0 X 10^{-2}M adenosine, the average production of *ade2-1/ade2-1* was 5.0 pfd and that of *ade2-1*/SM5 was 3.9 pfd. Similarly at the concentration of 3.2 X 10^{-3}M inosine, the average production was 5.3 pfd and 3.5 pfd respectively. Moreover, the heterozygotes, *ade2-1*/SM5, were also apparently resistant when compared with their counterparts, B82-4/SM5 or C42-6/SM5: At 1.0 X 10^{-3}M, the average production of *ade2-1*/SM5 was 4.1 pfd and 3.0 pfd on adenosine and guanosine respectively; that of B82-4 and C42-6/SM5 was 1.4 pfd and 2.1 pfd (adenosine) and, 0.8 pfd abd 4.4 pfd (guanosine). Although the last figure (4.4) is probably not different from 4.1, the other three (1.4, 2.1 and 0.8) are clearly low. Furthermore, the average production of *ade2-1*/SM5 is raised from 2.5 pfd on Sang's unsupplemented medium to 5.5 pfd on 1.0 X 10^{-2}M guanosine, despite the fact that guanosine does not supplement the *ade2-1/ade2-1* mutant. Given that, in general, non-toxic additives improve the average production of heterozygotes (see responses of *pyr2-1* and *pyr2-2* to pyrimidine precursors, below) and that guanosine is indeed toxic to wild-type flies (el Kouni, unpublished) it does not seem unreasonable to suggest that *ade2-1*/SM5 are resistant, not only to adenosine or inosine but to purine nucleosides in general.

Inspection of the average production on Sang's unsupplemented medium also shows a rise in *ade2-1*/SM5 heterozygotes from 2.5 pfd to 3.9

pfd and to 6.7 pfd on 1.0 X 10^{-2}M adenosine and inosine respectively. This may be an indication that not only the resistance to purines but also the nutritional requirement of *ade2-1* mutant is semidominant. Indeed, the production of *ade2-1* heterozygotes on Sang's unsupplemented medium is lower than that of C95-11 on the same medium. Average production in type 1 crosses were 2.2 pfd for *ade2-1* and 4.2 pfd for C95-11. In contrast, on yeast-sucrose medium, the equivalent figures were 4.7 pfd and 5.0 pfd.

ade2-1 was tested for temperature sensitivity and was found to grow well at both 29°C and 20°C.

The pyrimidine requirers B82-4 (*pyr2-1*) and C42-6 (*pyr2-2*)

The maternal effect

The conditional lethalities of B82-4 and C42-6 are maternally influenced. When grown on restrictive medium (Table 6c and d), the relative viabilities of homozygotes from type 1 crosses (homozygous ♀ X heterozygous ♂) was 1.8% for B82-4 and 2.4 for C42-6. In type 3 crosses (heterozygous ♀ X homozygous ♂) these figures rose to 22.9% and 37.8% respectively. The delay in development in unsupplemented medium was also maternally affected. B82-4 homozygotes were delayed (relative to the heterozygous segregants) by 11.0 days in type 1 crosses, but by 6.6 days in type 3 crosses. In C42-6, the corresponding delays were 7.8 days compared to 5.2 days.

In general, the maternal effect is enhanced whenever metabolites added to Sang's defined medium seemed not to supplement. Satisfactory supplements, on the other hand, mask the maternal effect.

Map position

Because crossing over in Drosophila has to be observed in female gametogenesis and since B82-4 and C42-6 exhibit a high level of leakiness in heterozygous mothers, difficulties might be expected with the mapping of these two mutants (see A66-17 above). Indeed, using total progeny, both B82-4 and C42-6 give negative values for recombination with *bw*^{D}. The recombination frequencies were, respectively, r =-0.04 and r =-0.79 g (Table 17). However, experience showed both B82-4 and C42-6 homozygotes from heterozygous mothers first appear five days later than the heterozygous segregants and it was possible to exploit this characteristic to map the two mutants. Using progeny emerging during the first five days of eclosion, it was found that the nutritional requirement of B82-4 maps at approximately 1.5 units from *bw* (r = 0.02) whilst that of C42-6 maps at ∿ 2.6 units from *bw* (r = 0.03) (Table 17). Given the vagaries of the method, these two locations are quite likely to represent mutations close enough together to affect the same gene locus, in accord with the conclusion derived from the complementation test. Since the best nutritional response of these two mutants seemed to be with pyrimidine ribonucleosides, this locus has been designated *pyr2* with two mutant alleles *pyr2-1* (B82-4) and *pyr2-2* (C42-6).

Dose response to purine ribonucleosides and to uridine

Supplementation of *pyr2-1* and *pyr2-2* by the four common ribonucleosides did not give clear cut results as to whether they were purine or pyrimidine requirers. In an attempt to solve this problem, the dose response of these two mutants to purine ribonucleosides and to uridine were investigated. Cytidine was ignored since its earlier effect paral-

Table 17: Mapping the nutritional requirement of B82-4 (*pyr2-1*) and C42-6 (*pyr2-2*)

Marker(s)	*S*			*Sp*			*J*			*Tft***			bw^D		
	S	S^+	*r*	*Sp*	Sp^+	*r*	*J*	J^+	*r*	*Tft*	Tft^+	*r*	bw^D	bw^{D+}	*r*
B82-4															
Total progeny	304 (428)	330 (529)*	0.52	205 (500)	430 (457)	0.59	136 (689)	453 (644)	0.25	303 (63)	288 (71)	0.38	619 (684)	120 (738)	-0.04
First five days progeny	156 (424)	151 (528)	0.49	124 (496)	183 (456)	0.50	119 (592)	325 (539)	0.60	230 (49)	185 (56)	0.38	448 (631)	7 (655)	0.02
C42-6															
Total progeny	360 (380)	414 (512)	0.63	297 (443)	477 (449)	0.59	174 (317)	299 (296)	0.43	205 (153)	269 (177)	0.85	585 (782)	157 (792)	-0.79
First five days progeny	157 (372)	191 (509)	0.54	158 (441)	190 (440)	0.45	141 (275)	206 (252)	0.44	156 (65)	183 (84)	0.55	553 (684)	15 (648)	0.03

	SSp			
	SSp	SSp^+	S^+Sp	S^+Sp^+
B82-4				
Total progeny	131 (299)	174 (129)	74 (201)	256 (328)
First five days progeny	78 (295)	78 (129)	46 (201)	105 (327)

	SSp			
	SSp	SSp^+	S^+Sp	S^+Sp^+
C42-6				
Total progeny	181 (270)	179 (110)	116 (173)	298 (339)
First five days progeny	92 (268)	65 (104)	66 (173)	125 (336)

* Number in parenthesis represents equivalent data on yeast-sucrose medium

** *s* derived from C42-6 in the case of B82-4 and *vice versa* lelled that of uridine. The results are presented in Table 18.

The most striking result of the dose response study is perhaps the fact that uridine, at a relatively low concentration, is still a more or less adequate supplement whilst adenosine and to a lesser extent guanosine seem to be quite inadequate even at doses higher than the lowest uridine concentration. For example, in type 2 crosses, the average production of *pyr2-1/pyr2-1* dropped from 11.0 pfd on 5.0 X 10^{-3}M uridine to 6.4 pfd on 5.0 X 10^{-4}M uridine (table $18c_1$) whilst on guanosine, it dropped from 10.3 pfd on 5.0 X 10^{-3}M to 0.9 pfd on 1.0 X 10^{-3}M and 0.4 pfd on 5.0 X 10^{-4}M (table $18b_1$). Equivalent figures are exhibited by *pyr2-2/pyr2-2* (table $18b_2$ and $18c_2$). The developmental time, on the other hand, seemed to be particularly sensitive to any decrease in the concentration of either uridine or purine ribonucleosides. In type 1 crosses, a similar but less extreme pattern was exhibited (see discussion of the effect of co-existence with heterozygous segregants above). The maternally influenced resistance of *pyr2-1* homozygotes to guanosine persisted at the lower concentration of 2.5 X 10^{-3}M.

Another striking characteristic of the dose response study is the fact that the average production of heterozygous mutants, with increasing concentrations of either adenosine or guanosine, roughly followed an inverted bell shape. This, together with the fact that the optimal dose for supplementation of *ade2-1* by adenosine and inosine was 1.0 X 10^{-2}M, led to the investigation of the response of *pyr2-1* and *pyr2-2* to higher doses than the standard 5.0 X 10^{-3}M, for either purine ribonucleosides. The results are presented in Table 19.

The concentration of 5.0 X 10^{-2}M adenosine or guanosine was highly

Table 13a$_1$: Dose response of *pyr2-1* (B82-4) to adenosine

Cross	$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{SM5}$ ♂								$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	7	403	0.2	9.0	24.3±4.1	13.2±1.1	1.7	11.1	45	1.0	22.2±2.3
5.0 X 10^{-4}M	1	163	0.02	3.6	-	12.7±1.1	0.6	-	6	0.1	18.3±1.2
7.5 X 10^{-4}M	2	216	0.04	4.8	13.3±2.1	12.2±1.2	0.9	1.1	11	0.2	18.9±3.4
1.0 X 10^{-3}M	2	61	0.04	1.4	14.0±1.4	12.2±1.2	3.3	1.8	4	0.08	17.5±1.9
2.5 X 10^{-3}M	2	51	0.04	1.1	17.5±2.1	11.5±1.2	3.9	6.0	34	0.8	16.8±2.0
5.0 X 10^{-3}M	34	163	0.8	3.6	13.4±1.5	11.4±1.1	20.9	2.0	131	2.9	13.1±1.7
5.0 X 10^{-3}M*	129	234	2.9	5.2	12.9±1.2	11.8±1.3	55.1	1.1			

* Results of the reciprocal cross $\frac{pyr2\text{-}1}{SM5}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ o (type 3 crosses)

Table 18a$_2$: Dose response of *pyr2-2* (C42-6) to adenosine

Cross	$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ x $\frac{pyr2\text{-}2}{SM5}$ ♂								$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ x $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	7	449	0.1	7.4	17.6±2.8	13.7±2.1	1.6	3.9	9	0.3	21.7±3.0
5.0 X 10^{-4}M	3	100	0.1	4.2	20.0±2.1	12.9±1.6	3.0	7.1	9	0.3	18.2±2.5
7.5 X 10^{-4}M	4	98	0.08	2.0	14.9±4.3	12.8±1.6	4.1	2.1	13	0.5	16.5±4.1
1.0 X 10^{-3}M	5	51	0.2	2.1	15.8±2.9	12.3±1.7	9.8	3.5	16	0.7	14.6±2.3
2.5 X 10^{-3}M	36	105	1.5	4.4	14.7±3.7	13.1±2.0	34.3	1.6	21	0.9	13.0±2.7
5.0 X 10^{-3}M	29	47	1.2	2.0	13.3±2.2	11.3±1.5	61.7	2.0	26	2.2	14.3±3.8

Table $18b_1$: Dose response of *pyr2-1* (B82-4) to guanosine

Cross	$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ X $\frac{pyr2\text{-}1}{SM5}$ ♂								$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ X $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	7	403	0.2	9.0	24.3±4.1	13.2±1.1	1.7	11.1	45	1.0	22.2±2.3
5.0×10^{-4}M	2	122	0.04	2.7	20.0±0.0	12.2±1.2	1.6	7.8	16	0.4	20.7±1.9
7.5×10^{-4}M	4	118	0.09	2.6	18.0±4.7	12.1±1.2	3.4	5.9	9	0.2	19.6±1.7
1.0×10^{-3}M	5	35	0.1	0.8	18.2±2.8	12.4±1.1	14.3	5.8	39	0.9	19.7±1.6
2.5×10^{-3}M	77	51	1.7	1.1	18.5±2.1	12.6±1.1	151.0	5.9	157	3.5	17.8±2.0
5.0×10^{-3}M	152	77	3.4	1.1	16.9±2.0	13.3±2.0	197.4	3.6	465	10.3	18.4±2.4
5.0×10^{-3}M *	119	249	2.6	5.5	17.7±1.9	14.1±1.8	47.8	3.6			

* Results of the reciprocal cross (type 3 crosses)

Table 18b$_2$: Dose response of *pyr2-2* (C42-6) to guanosine

Cross	$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{SMS}$ ♂								$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂		
	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$	$\frac{m}{m}$	$\frac{m}{SMS}$					
None	7	449	0.1	7.4	17.6±2.8	13.7±2.1	1.6	3.9	9	0.3	21.7±3.0
5.0 X 10^{-4}M	6	243	0.1	5.1	15.6±5.0	13.2±1.5	2.5	2.4	5	0.1	17.4±2.7
7.5 X 10^{-4}M	11	116	0.3	3.2	18.3±2.7	13.2±1.9	9.5	5.1	16	0.4	19.6±2.8
1.0 X 10^{-3}M	14	160	0.4	4.4	20.4±2.7	13.2±1.6	8.9	7.2	17	0.7	16.9±3.1
2.5 X 10^{-3}M	17	29	1.4	2.4	18.1±2.8	12.7±2.2	58.6	5.3	98	2.7	18.1±2.8
5.0 X 10^{-3}M	19	49	0.8	2.0	17.5±2.9	13.2±2.0	38.8	4.3	104	4.3	19.6±3.1

Table 18c$_1$: Dose response of *pyr2-1* (B82-4) to uridine

Cross	$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{SM5}$ ♂								$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂		
	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	2	296	0.04	6.2	26.0±6.4	13.0±1.4	0.7	13	20	0.4	22.8±2.4
5.0×10^{-4}M	50	358	1.0	7.5	21.1±2.6	12.6±1.5	14.0	8.5	305	6.4	19.2±1.9
7.5×10^{-4}M	118	349	3.3	10.0	20.2±2.8	12.8±1.9	33.8	7.4	633	10.4	19.5±1.8
1.0×10^{-3}M	122	347	3.4	9.6	20.0±1.9	12.8±1.5	35.0	7.2	572	11.9	18.6±2.3
2.5×10^{-3}M	374	410	6.2	6.8	17.9±2.4	13.2±1.3	91.2	4.7	682	11.4	17.3±2.1
5.0×10^{-3}M	402	473	8.4	9.9	16.8±2.2	13.4±1.8	85.0	3.4	529	11.0	16.2±2.0
5.0×10^{-3}M *	366	535	6.1	8.9	15.7±1.6	13.5±1.5	68.4	2.2			

* Results of the reciprocal cross (type 3 crosses)

Table 18c$_2$: Dose response of *pyr2-2* (C42-6) to uridine

Cross	$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{SM5}$ ♂								$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	10	352	0.2	5.9	26.1±2.1	13.0±1.5	2.8	13.1	35	0.7	20.4±1.7
5.0 X 10^{-4}M	171	401	2.9	6.7	22.3±2.4	14.5±1.7	42.9	7.8	343	5.7	19.1±1.9
7.5 X 10^{-4}M	250	470	4.2	7.8	20.5±2.2	13.0±1.4	53.2	7.5	747	12.5	18.7±2.1
1.0 X 10^{-3}M	256	420	4.3	7.0	19.6±2.2	13.4±1.4	61.0	6.2	850	14.2	20.2±2.5
2.5 X 10^{-3}M	358	553	6.0	9.2	17.4±2.1	13.4±1.5	64.7	4.0	851	14.2	15.7±2.1
5.0 X 10^{-3}M	386	515	6.4	8.6	16.3±2.2	13.5±1.9	75.0	2.7	1127	18.8	15.1±2.0
5.0 X 10^{-3}M *	406	553	6.8	9.2	17.2±2.3	14.4±2.2	73.4	2.9			

* Results of the reciprocal cross (type 3 crosses)

Table 19; Response of *pyr2-1* and *pyr2-2* to 1.0 X 10^{-2}M and 5.0 X 10^{-2}M adenosine and guanosine

Cross		$\frac{mutant}{mutant}$ ♀ x $\frac{mutant}{SM5}$ ♂								$\frac{mutant}{mutant}$ ♀ x $\frac{mutant}{mutant}$ ♂		
		Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Strain	Supplement	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
pyr2-1	None	0	323	0	7.2	-	14.8±1.1	0.0	-	13	0.3	22.6±2.7
	AR^a	144	140	3.1	3.2	14.3±2.1	13.1±1.4	10[illegible]9	1.2	257	5.7	14.4±1.3
	AR^b	3	38	0.07	0.8	22.0±0.0	19.0±2.0	7.9	3.0	13	0.3	22.5±1.3
	GR^a	38	214	1.1	5.9	19.2±1.9	15.2±1.8	17.8	4.0	238	5.3	19.4±2.0
	GR^b	0	0	0	0	-	-	0.0	-	1	0.02	-
pyr2-2	None	0	168	0	3.7	-	14.6±1.4	0.0	-	2	0.04	17.0±2.1
	AR^a	37	65	0.8	1.4	15.9±1.6	13.4±1.1	56.9	2.5	77	1.7	15.7±1.7
	AR^b	0	6	0	0.1	-	18.5±1.2	0.0	-	0	0	-
	GR^a	41	111	0.9	2.5	20.0±2.1	15.0±1.6	36.9	5.0	111	2.5	20.5±2.2
	GR^b	1	2	0.02	0.04	-	22.0±1.4	50.0	-	7	0.2	18.2±1.3

a = 1.0 X 10^{-2}M

b = 5.0 x 10^{-2}M

toxic to *pyr2-1* and even more so to *pyr2-2*. On 1.0 X 10^{-2}M adenosine, type 1 crosses exhibited a relative viability of 102.9% for *pyr2-1* and 56.9% for *pyr2-2*. The developmental delay for *pyr2-1* was 1.2 days and for *pyr2-2* 2.5 days. On 1.0 X 10^{-2}M guanosine, in contrast, the relative viability of *pyr2-1* was 17.8%, that of *pyr2-2* was 36.9%, and the developmental delay 4.0 days and 5.0 days respectively.

The results suggest that supplementation by both adenosine and guanosine is roughly equivalent; however, it seems that the optimum concentrations for adenosine is 1.0 X 10^{-2}M whereas that for guanosine is 5.0 X 10^{-3}M. However, the dosage studies do not unequivocally alter the earlier conclusion that uridine is a more adequate supplement than either purine nucleoside.

Response to intermediates in de novo pyrimidine biosynthesis

Dose response of both *pyr2-1* and *pyr2-2* to the purine ribonucleosides and to uridine confirmed the conclusion that the pyrimidine ribonucleosides supplement better than either guanosine or adenosine. At this point, it was decided that investigation of the response of the two alleles to pyrimidine precursors from the orotate pathway might give a clue as to the primary lesion in *pyr2-1* and *pyr2-2*. The results are presented in Table 20.

None of the precursors supplemented well. In type 1 crosses the relative viability of homozygotes was 4.5% on Sang's unsupplemented medium. The range of results on precursor supplemented media was from 10.9% on carbamyl phosphate to 16.9% on carbamyl aspartate with *pyr2-1*. With *pyr2-2* the equivalent figures were 6.0% on Sang's medium and from

Table 20a: Response of *pyr2-1* (B82-4) to the various pyrimidine precursors

Cross $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{SM5}$ ♂

Supplement (5.0 X 10^{-3}M)	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)
	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$		
None	8	177	0.1	3.0	22.4±4.0	13.6±1.4	4.5	8.9
Carbamyl phosphate	27	248	0.5	4.1	21.3±4.0	19.0±2.3	10.9	2.3
Carbamyl aspartate	46	273	0.8	4.6	25.2±3.6	15.3±1.4	16.9	9.8
Dihydro-orotate	47	392	0.8	6.5	25.8±2.2	15.7±2.0	12.0	10.1
Orotate	40	368	0.7	6.1	26.4±2.4	15.8±1.7	10.9	10.6
Uridine	286	331	4.8	5.5	17.8±1.5	15.3±1.8	86.4	2.4

Cross	$\frac{pyr2\text{-}1}{SM5}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂								$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
Supplement (5.0×10^{-3}M)											
None	107	372	1.2	612	20.9±2.2	14.4±2.2	28.8	6.5	12	0.2	22.7±1.7
Carbamyl phosphate	79	315	1.3	5.3	23.2±3.1	19.7±2.0	25.1	3.5 *	37	0.6	25.6±2.3
Carbamyl aspartate	138	428	2.3	7.1	23.3±2.5	16.7±1.7	32.2	6.6	82	1.4	26.8±2.2
Dihydro-orotate	163	487	2.7	8.1	23.4±2.4	16.4±2.0	33.5	6.9	71	1.2	25.4±2.2
Orotate	194	392	3.2	6.5	23.5±2.5	16.3±1.9	49.5	7.2	16	0.3	26.2±2.2
Uridine	421	531	7.0	8.9	17.5±1.5	15.7±2.0	79.3	1.8	515	8.9	17.5±1.6

* Note that these values are smaller because the development of the heterzygous segregants is delayed

Table 20b: Responses of *pyr2-2* (C42-6) to the various pyrimidine precursors

Cross $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{SM5}$ ♂

Supplement (5.0 X 10^{-3}M)	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)
	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$		
None	13	217	0.2	3.6	21.6±5.0	13.6±1.4	6.0	8.1
Carbamyl phosphate	46	211	0.8	3.5	23.5±3.8	18.7±2.1	21.8	4.8
Carbamyl aspartate	84	238	1.4	4.0	25.6±2.8	15.3±1.9	35.3	10.3
Dihydro-orotate	68	252	1.1	4.2	25.6±2.9	15.8±1.6	27.0	9.9
Orotate	45	127	0.8	2.1	27.2±2.5	16.1±1.5	35.4	11.2
Uridine	280	303	4.7	5.1	16.6±1.6	14.9±1.5	92.4	1.7

Cross	$\frac{pyr2\text{-}2}{SM5}$ ♀ x $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂								$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ x $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂		
	Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Supplement ($5.0 \times 10^{-3}M$)	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	149	385	2.5	6.4	19.6±2.1	13.8±1.6	39.0	5.8	34	0.6	21.3±2.2
Carbamyl phosphate	98	327	1.6	5.5	22.7±2.8	18.7±1.9	30.0	4.0	106	1.8	25.7±2.4
Carbamyl aspartate	144	559	2.4	9.3	22.5±3.0	16.1±2.0	25.8	6.4	172	2.9	24.8±2.7
Dihydro-orotate	135	420	2.8	8.9	22.5±2.3	16.2±1.8	32.1	6.3	76	3.2	26.7±2.2
Orotate	178	478	3.0	8.0	22.0±2.2	16.1±2.0	37.2	5.9	26	1.1	26.3±2.4
Uridine	194	296	4.0	6.2	16.1±1.6	14.8±1.8	65.5	1.3	550	9.2	17.1±1.6

21.8% to 35% on the precursors. The developmental delay on most precursors was approximately ten days for both *pyr2-1* and *pyr2-2*. In type 3 crosses, the relative viability of homozygotes rose to 28.8% on Sang's medium and ranged from 25.1% on carbamyl phosphate to 49.5% on orotate for *pyr2-1;* similar results were found for *pyr2-2*. These results show that a strong maternal effect persists on precursors supplemented media, except on carbamyl phosphate, where a smaller developmental delay and a lack of maternal effect are, apparently, a result of a delay in the development of heterozygous segregants.

None of the precursors exhibited a toxic effect, as measured by the production of *mutant*/SM5 offspring. For example, in type 1 crosses, the production of *pyr2-1* heterozygotes was 3.0 pfd on Sang's unsupplemented medium and ranged from 4.1 pfd on carbamyl phosphate to 6.5 pfd on dihydroorotate, with more or less equivalent figures for *pyr2-2* and the reciprocal cross (type 3 crosses).

Most precursors were not much different from uridine in their effect upon the development time of controls; uridine itself, however, increases the developmental time of *mutant*/SM5 flies approximately a day relative to their development on Sang's unsupplemented medium. Production of heterozygous mutants, on the other hand, shows a slight improvement on uridine relative to Sang's unsupplemented medium (5.5 pfd vs 3.0 pfd and 5.1 pfd vs 3.6 pfd for *pyr2-1* and *pyr2-2* respectively). With *ade2-1*, a similar but stronger effect on adenosine has been taken as an indication of a possible semi-dominant nutritional requirement. With *pyr2-1* and *pyr2-2* this speculation is not favoured, since in addition to uridine pyrimidine precursors, which do not supplement homozygotes do increase production of heterozygous mutants. Moreover, the average production of either *pyr2-1* or *pyr2-2* heterozygotes from type 1 crosses on Sang's unsupplemented medium is equivalent to that of C95-11 heterozygotes generated under the same conditions (5.2 pfd and 5.2 pfd vs 5.3 pfd). It is speculated that the increase in *mutant*/SM5 production in the case of supplementation with the pyrimidine precursors results from overriding the rate limiting properties of carbamyl phosphate synthetase (CPSase) (Jarry and Falk, 1974).

Carbamyl phosphate is exceptional, in so far as it delays both mutant and heterozygous development, regardless of whether the female parents were homozygous or heterozygous. Carbamyl phosphate is negatively charged and would not be expected to permeate cell membranes easily. Moreover, it is unstable. Thus its adequacy as a potential supplement is questionable. However, its capacity to delay development of control segregants or to increase their production indicates that it (or its derivatives) has some pharmacological effect.

In type 2 crosses (homozygous ♀ X homozygous ♂) particularly, the production of *mutant/mutant* flies on orotate supplemented medium is less than that on carbamyl aspartate or dihydroorotate.

In contrast to supplementation with either pyrimidine or purine ribonucleosides, which shortened the developmental time of the homozygous mutants, most precursors seem to cause at least a further 24 hours delay over the considerable delay observed with flies grown on Sang's unsupplemented medium. Undoubtedly, the delay observed in the mutants when grown on Sang's unsupplemented medium must be caused by a slowing down of growth processes.

With the pyrimidine precursors, the question arises as to whether the small changes identified as alterations of developmental rate, actually represent changes in the average behaviour of flies or result from changes in the spectrum of surviving flies; for instance, when a delay in development is found, is this because all the flies developed more slowly or because a number of slower developing flies were able to survive under the altered conditions? Mutant larvae which are destined to die on unsupplemented cultures remain small but alive for a week or more. Addition of the precursors prolongs this phenomenon. If this delay in the onset of mortality were throughout the population of the larvae, an effect of this latter kind might result. The effect is not necessarily supplementation, since larval growth is still radically disturberd, but might also represent a non-specific amelioration of the developmental environment. If such an effect were occurring, there should be a positive correlation between the relative viability of homozygous mutants and their developmental time on the various precursors. Such correlation exists for type 2 crosses, but is absent in type 1 and type 3 crosses, in which control segregants coexist with the mutant. Clearly these segregants modify the culture conditions, so that the absence of correlation is not critical.

It is concluded that despite the fact that the average production of homozygous mutants on some pyrimidine precursors is equivalent to that on adenosine, the least effective riboside, the precursors are inadequate supplements because they do not improve the developmental rate which, seems to indicate that they fail to provide the metabolite which is required by the mutants.

Orotidine is a by-product of the *de novo* pyrimidine biosynthesis. Although the enzyme orotidine kinase is not mentioned in the literature, *in vitro* preparation of orotidylate from orotidine is reported (Lieberman, *et. al.* 1955). Therefore, an attempt was made at using orotidine as a supplement. The results are reported in Table 21.

Since the standard dose of 5.0 X 10^{-3}M proved completely lethal to both homozygous and heterozygous mutants, lower doses were tested. The concentration of 3.2 X 10^{-3}M was still very toxic for both *pyr2-1* and *pyr2-2* heterozygotes. However, with *pyr2-1*, lower concentrations, 1.0 X 10^{-3}M and particularly 1.0 X 10^{-4}M, improved the internal controls (*mutant*/SM5); the former also allowed a slight increase in the average production of homozygous mutants (from 0.03 pfd on unsupplemented medium to 0.3 pfd on orotidine). This increase is also obvious in type 2 crosses where the equivalent figures were 0.3 pfd and 1.2 pfd. Except at the doses that proved partially lethal to the heterozygotes, no delay accompanied the growth of heterozygous mutants. Homozygous survivors, on the other hand, were extemely delayed (9.2 - 13.4 days). With *pyr2-2* the same pattern was exhibited with higher sensitivity to orotidine among the heterozygous mutants, low level "supplementation" at the concentration of 1.0 X 10^{-4}M for homozygous mutants and an even more extreme delay in the development of the homozygotes (14.1 - 16.7 days).

As a control, orotidine was tested as a supplement for three different rudimentary stocks, namely $r^{pyr1\text{-}1}$, $r^{pyr1\text{-}10}$, and $r^{pyr1\text{-}19}$; (Table 21c) It was concluded that orotidine is not utilizable for *de novo* biosynthesis of pyrimidines in *D. melanogaster*, since it failed to supplement these mutants, whose primary lesion is known to be in the early

Table 21a: Dose response of *pyr2-1* (B82-4) to orotidine

Cross	$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{SM5}$ ♂								$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂		
	Number of Progeny		Average Pro- duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro- duction	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	2	328	0.03	5.5	21.5±5.6	12.6±1.4	0.6	8.9	15	0.3	22.0±2.5
1.0 X 10^{-5}M	3	382	0,05	6.4	21,8±5.1	12.6±1.4	0.8	9.2	11	0.2	24.2±2.2
1,0 X 10^{-4}M	4	580	0.07	9.7	26.0±1.7	12.6±1.5	0.7	13.4	20	0.3	22.5±1.9
1.0 X 10^{-3}M	17	435	0.3	7.3	24.8±2.6	13.8±1.9	3,9	11.0	73	1.2	24.1±2.6
3.2 X 10^{-3}M	0	100	0	1.7	-	17.5±2.5	0.0	-	2	0.03	28.5±4.2

Table 21b: Dose response of *pyr2-2* (C42-6) to orotidine

Cross	$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{SM5}$ ♂								$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ X $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂		
	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Concentration	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
None	10	357	0.2	6.0	26.5±1.7	13.2±1.4	2.8	13.3	75	1.3	25.2±1.6
1.0 X 10^{-5}M	5	346	0.02	5.8	27.5±1.4	13.4±1.6	1.5	14.1	16	0.3	24.7±1.6
1.0 X 10^{-4}M	16	394	0.3	6.6	29.8±1.7	13.1±1.3	4.1	16.7	57	1.0	24.5±1.8
1.0 X 10^{-3}M	3	272	0.02	4.5	29.2±2.1	14.8±2.2	1.1	14.4	11	0.2	26.8±1.9
3.2 X 10^{-3}M	0	22	0	0.4	-	18.0±1.5	0.0	-	0	0	-

Table 21c: Response of $r^{pyr1\text{-}1}$, $r^{pyr1\text{-}10}$, and $r^{pyr1\text{-}19}$ to orotidine

Supplement (5.0 X 10^{-3})	Phenotype	Number of Progeny	Average Production	Developmental Time Mean ± S.D.	% Rel. Viab.
			$r^{pyr1\text{-}1}$		
None	r/Y	31	0.1	17.4±2.0	8.5
	$\widehat{XX}$/Y	364	1.5	15.0±1.5	
OR	r/Y	120	0.5	19.4±2.2	30.0
	$\widehat{XX}$/Y	400	1.7	16.9±2.4	
UR	r/Y	555	2.3	17.5±1.7	113.0
	$\widehat{XX}$/Y	491	2.1	15.6±1.5	
			$r^{pyr1\text{-}10}$		
None	r/Y	0	0	-	0.0
	$\widehat{XX}$/Y	360	1.5	16.0±1.6	
OR	r/Y	0	0	-	0.0
	$\widehat{XX}$/Y	182	0.8	19.0±1.8	
UR	r/Y	203	0.9	16.0±1.6	104.6
	$\widehat{XX}$/Y	194	0.8	16.2±1.7	
			$r^{pyr1\text{-}19}$		
None	r/Y	0	0	-	0.0
	$\widehat{XX}$/Y	188	0.8	14.8±1.4	
OR	r/Y	0	0	-	0.0
	$\widehat{XX}$/Y	196	0.8	16.4±1.9	
UR	r/Y	43	0.2	16.2±1.1	16.4
	$\widehat{XX}$/Y	263	1.1	14.9±1.2	

steps of pyrimidine biosynthesis.

None of the pyrimidine precursors supplemented well, and since pyrimidine ribonucleosides showed appreciable supplementation, it was concluded that, if they are defective in pyrimidine biosynthesis, *pyr2-1* and *pyr2-2* must be deficient in either or both orotate phosphoribosyltransferase (OPRTase) and orotidylate decarboxylase (ODCase). Supplementation by purine nucleosides was speculated to be a result of their acting as ribose-1-Pdonors, which would recycle uracil into uridylate, thus, if not completely substituting for *de novo* pyrimidine biosynthesis, at least relieving some of the ill effects of a limited biosynthetic capacity.

Enzymology

The conversion of C^{14}-orotate to uridine-5'-monophosphate (UMP), uridine (UR) and uracil (U) was used as an assay for the activities of both OPRTase and ODCase. Orotidine-5'-monophosphate (OMP), an intermediate in production of uridylate from orotate, is apparently converted immediately to uridylate, since it could not be chromatographically identified. The results are shown in Table 22 and fig. 7.

The two mutants exhibited appreciable levels of activity which are quite incompatible with the hypothesis that the pyrimidine requirement is caused by a deficiency in either or both OPRTase and ODCase. Moreover, the activities of these two enzymes seem to be 2 - 3 times higher than the activities in the control strain C95-11, when the enzyme extract is taken from larvae grown on Sang's unsupplemented and guanosine supplemented media. However, it would seem that the enzyme activities

Table 22: Conversion of ^{14}C - orotate (nannomoles/mg protein) to UMP, UR and U in assays of OPRTase activity

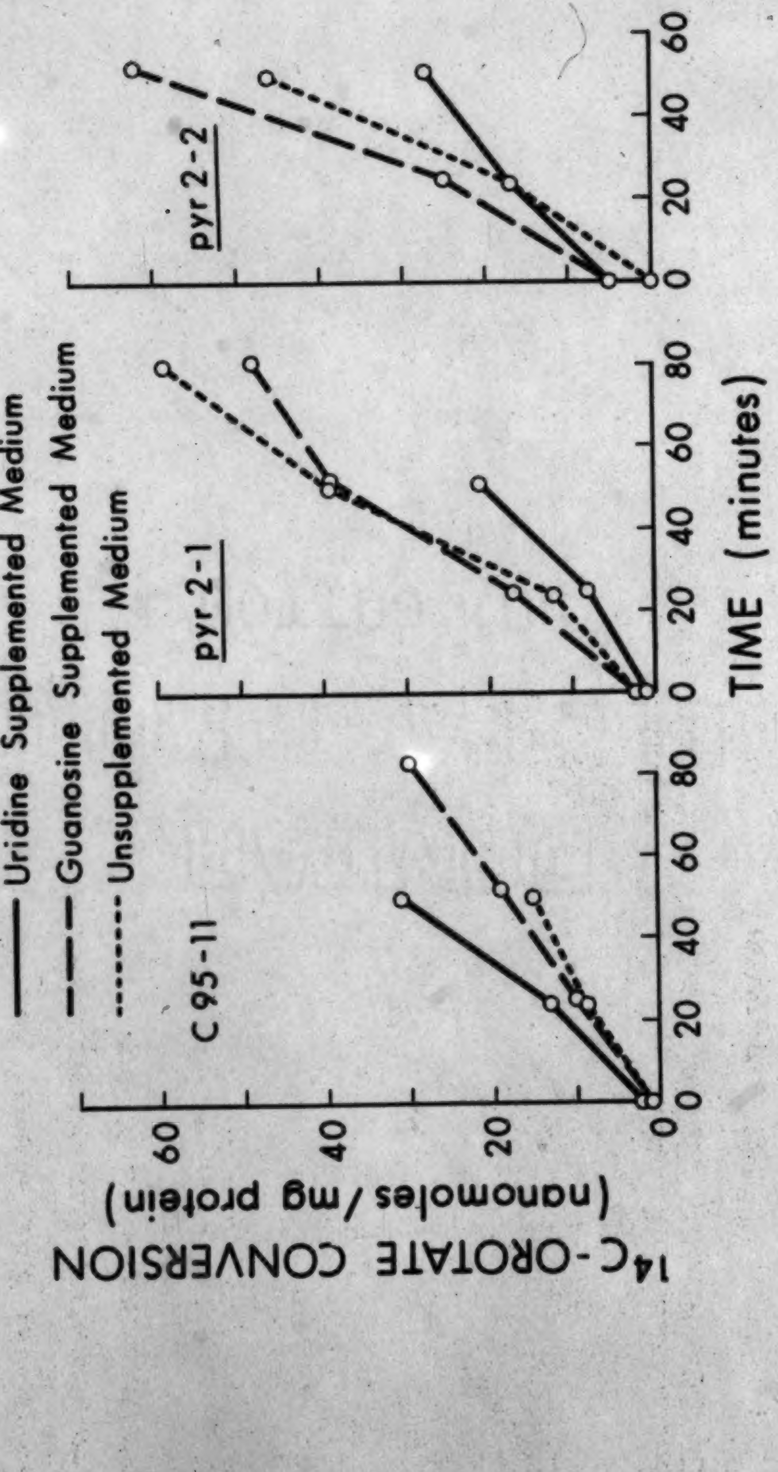
Time (minutes)		0			25			50			80		
		Media			Media			Media			Media		
Metabolite	Strain	Sang's	+UR	+GR	Sang's	+UR	+GR	Sang's	+UR	+GR	Sang's	+UR	+GR
UMP + UR + U	C95-11	0.997	1.993	1.157	8.985	13.052	10.388	14.576	31.434	18.595	-	-	30.085
	pyr2-1	2.026	0.947	1.661	16.887	7.528	17.002	39.181	20.984	38.530	58.650**	-	48.022**
	pyr2-2	-	4.883	4.653	16.501	17.193	24.636	46.099	27.251	62.099	-	-	-
UR + U	C95-11	0.212	-	-	6.475	7.379	8.292	11.728	24.498	15.729	-	-	27.668
	pyr2-1	-	0.326	-	13.662	5.716	13.151	35.558	19.709	34.695	58.650		48.022
	pyr2-2	-	1.769	-	13.301	13.059	17.180	42.728	22.564	53.721	-	-	-
U	C95-11	-	-	-	-	-	1.143	-	*	1.752	-	-	3.135
	pyr2-1	-	-	-	-	-	0.701	-	1.275	2.842	5.801	-	4.172
	pyr2-2	-	-	-	-	-	-	4.778	-	2.419	-	-	-

* Both uridine and uracil counted on same cut because their spots overlapped

** Orotate completely converted to uridine and uracil

OPRTase rather than ODCase must be rate limiting since OMP does not show up as a spot on the chromatograms. Since, initially, the counts decrease from UMP to UR and from UR to U, the conversion sequence is probably orotate → UMP → UR → U, as expected. Therefore, the amount of UMP formed must be represented by the sum of counts in UMP, UR and U. Similarly, conversion of UMP to UR must be represented by the sum of the counts found in UR and U.

Figure 7 . Conversion of ^{14}C-orotate to orotidylate by extracts from larvae grown on Sang's defined medium. The activity of orotate phosphoribosyl transferase was estimated from the total production of uridylate, uridine and uracil. No orotidylate was observed, presumably because OMP-decarboxylase activity is present in excess in the crude extracts.


Uridine Supplemented Medium
Guanosine Supplemented Medium
Unsupplemented Medium
C 95-11
pyr 2-1
pyr 2-2
^{14}C-OROTATE CONVERSION (nanomoles / mg protein)
0
20
40
60
0
20
40
60
80
0
20
40
60
80
0
20
40
60
TIME (minutes)

in larvae grown on uridine are about the same as that of the control C95-11. The control strain shows a similar level of activities whether the extract is taken from larvae grown on uridine, guanosine or Sang's unsupplemented medium.

Higher enzyme activities in the mutants relative to the control strain and repression of the enzyme in the presence of uridine could arise in two ways: the mutants may have an elevated OPRTase activity resulting in a depletion of the PRPP pool which would culminate in lethality under restrictive conditons. Exogenous uridine may repress OPRTase thus leading to a normal PRPP pool and larval survival. Alternatively, the mutant may be deficient in PRPP biosynthesis resulting in starvation for UMP which would cause the elevated enzyme activity. Exogenous uridine, by supressing the starvation for UMP, brings back OPRTase synthesis to normal. In either case a low PRPP pool results in lethality, supplementation with uridine corrects the defect.

Supplementation by uracil

In order to gain further insight into the problem, it was decided to investigate supplementation by uracil. This metabolite is not part of the main pathway of *de novo* pyrimidine biosynthesis yet it is clearly related to uridine which is a good supplement. Moreover, it is utilizable by *Drosophila* since it was shown by Norby (1970) to adequately supplement *rudimentary*. Furthermore, as orotate, it is a PRPP receptor that in the presence of uracil phosphoribosyl-transferase is converted to uridylate (Reyes, 1969; Reyes and Hall, 1969 and Jund and LaCroute, 1972). The results are presented in Table 23.

Table 23: Response of *pyr2-1* and *pyr2-2* to uracil

Cross	$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{SM5}$ ♂								$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{mutant}$ ♂		
	Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Strain Supplement (5.0 X 10^{-3}M)	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
pyr2-1											
None	3	332	0.05	5.5	20.2±1.5	13.8±1.8	0.9	6.3	7	0.1	21.8±2.4
U	37	595	0.6	9.9	22.8±2.8	14.5±2.3	6.2	8.2	44	0.7	24.2±2.7
UR	228	332	3.8	5.5	17.2±1.8	13.9±1.4	68.7	3.3	657	10.9	16.5±1.7
pyr2-2											
None	1	373	0.02	6.2	-	13.9±1.5	0.3	-	27	0.5	18.3±2.8
U	40	468	0.7	7.8	24.3±2.9	14.2±1.6	8.6	10.1	416	6.9	20.7±2.7
UR	307	500	5.1	8.3	16.9±1.9	14.3±1.7	61.4	2.6	683	11.4	15.4±2.0

Uracil does not seem to supplement either *pyr2-1* or *pyr2-2* mutants. The relative viabilities of homozygous mutants from type 1 crosses were respectively 6.2% and 8.6% as opposed to 0.9% and 0.3% on Sang's unsupplemented medium. The developmental delay was 8.2 days and 10.1 days compared with 6.3 days on Sang's unsupplemented medium.

An anomolous result was found with *pyr2-2* homozygotes from type 2 crosses where the average production was 6.9 on uracil as opposed to 0.5 on restrictive medium. This incongruity has not yet been explained satisfactorily. It may be erroneous. However, the cultures were tested for possible infection and were found to be axenic. Moreover, the mutants did show an extreme delay characteristic of inadequate supplementation. The failure of uracil to support the growth of either *pyr2-1* or *pyr2-2* mutants was taken as evidence of the existence of a low pool of phosphoribosyl pyrophosphate.

Supplementation by purines

Since the experiments with uracil were performed, Reyes and Guganig (1975) have reported evidence that in animal cells, the same enzyme, pyrimidine phosphoribosyl-transferase catalyses the reactions

$$\text{PRPP + orotate} \xrightarrow{\text{PPRTase}} \xrightarrow{\text{ODCase}} \text{UMP}$$

$$\text{and PRPP + uracil} \xrightarrow{\text{PPRTase}} \text{UMP}$$

thus weakening the support that inadequate supplementation by uracil has given to the hypothesis of low phosphoribosyl pyrophosphate pools. Therefore, it was decided to investigate the supplementation pattern by purine bases which, like uracil, are unribosylated. Lack of supplementation by these compounds would reinforce the low PRPP pool hypothesis.

Adenine, shown to be utilizable by various auxotrophs (Johnson, unpublished) and guanine have been tested as potential supplements. The latter may not be utilizable by auxotrophs (Nash, unpublished). The results are pesented in Table 24. Supplementation by inosine was used as a novel control, since it is ribosylated, had not previously been tested and can be used by *Drosophila* (see *ade2-1*, for example).

Neither adenine nor guanine seems to supplement. In type 1 crosses the relative viability of *pyr2-1* on 1.0 X 10^{-3}M adenine was 0%. On 5.0 X 10^{-3}M adenine, it became 19.4%. It is to be noted, however, that the latter concentration of adenine is quite toxic. The average production of heterozygous mutants was 2.2 pfd and 0.7 pfd respectively as opposed to 5.3 pfd on Sang's unsupplemented medium, so that the comparatively high relative viability is quite possibly a function of the sensitivity of the heterozygotes (see interpretation of supplementation by nucleosides, above). On 1.0 X 10^{-3} guanine the relative viability was 0.5% and on 5.0 X 10^{-3}M guanine, it was 1.6%, the average production of heterozygous mutants being 8.3 pfd and 6.7 pfd respectively. The developmental delay on 5.0 X 10^{-3}M adenine was 7.7 days and on 1.0 X 10^{-3}M and 5.0 X 10^{-3}M guanine, it was 6.4 days and 8.9 days, respectively. The behaviour of *pyr2-1* on type 2 crosses parallels its behaviour on type 1 crosses.

In contrast, the supplementation by inosine is equivalent to adenosine at the same concentration (5.0 X 10^{-3}M). The relative viability of *pyr2-1* was 49.1%, the developmental delay 1.4 days and the average production 1.9 pfd.

Table 24: Response of *pyr2-1* and *pyr2-2* to 1.0 X 10^{-3}M and 5.0 X 10^{-3}M adenine and guanine and 5.0 X 10^{-3}M inosine

Cross		$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{SM5}$ ♂								$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{mutant}$ ♂		
		Number of Progeny		Average Production		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Production	Developmental Time Mean ± S.D.
Strain	Supplement	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
pyr2-1	None	0	256	0	5.3	-	15.0±1.8	0.0	-	19	0.4	26.6±1.8
	A^a	0	105	0	2.2	-	13.6±1.4	0.0	-	2	0.04	17.0±2.1
	A^b	6	31	0.1	0.7	21.3±2.3	13.6±1.6	19.4	7.7	2	0.04	15.5±0.0
	G^a	2	396	0.04	8.3	22.5±1.4	16.1±1.7	0.5	6.4	25	0.5	24.3±2.9
	G^b	5	321	0.1	6.7	24.7±1.3	15.8±1.5	1.6	8.9	18	0.4	25.5±2.9
	HR^b	56	114	0.9	1.9	15.5±1.3	14.1±1.4	49.1	1.4	211	3.5	15.1±1.9
pyr2-2	None	0	55	0	1.2	-	15.4±2.0	0.0	-	0	0	-
	A^a	0	34	0	0.9	-	13.6±1.8	0.0	-	1	0.02	-
	A^b	1	28	0.02	0.6	-	14.8±1.9	3.6	-	3	0.06	26.5±2.3
	G^a	1	133	0.02	2.8	-	15.4±2.1	0.8	-	2	0.04	30.5±0.0
	G^b	0	164	0	3.4	-	16.4±1.7	0.0	-	7	0.2	18.2±1.3
	HR^b	18	51	0.3	0.9	15.8±2.2	13.4±1.6	35.3	2.4	27	0.6	16.0±1.6

a = 1.0 X 10^{-3}M
b = 5.0 X 10^{-3}M

Results with *pyr2-2* were in agreement with *pyr2-1*.

It is concluded that both pyrimidine requirers can only be supplemented by the ribosylated nucleosides which in turn, suggests a low phosphoribosyl pyrophosphate pool in the mutants.

Responses to double supplementation with orotate, guanosine and uridine

A number of tests were run, utilizing double supplements. These tests were germane to hypotheses which have since been discarded. However, more recent hypotheses suggest an explanation for an unexpected outcome of some of these tests, so that the results are reported here in Table 25.

The "deleterious" effects of guanosine on homozygotes are not overcome by uridine; the average production of *mutant/mutant* flies in type 1 crosses was 2.3 pfd on guanosine, 2.1 pfd on guanosine and uridine and 8.4 pfd on uridine for *pyr2-1*. For *pyr2-2*, the equivalent figures were 2.5 pfd, 1.7 pfd, and 6.4 pfd, respectively. Similarly, the developmental times for *pyr2-1* were 18.1 days, 18.1 days and 16.8 days. For *pyr2-2* these figures were 17.4 days, 19.5 days and 16.3 days. Heterozygous segregants from type 1 crosses, both segregants in type 3 crosses and the progeny in type 2 crosses exhibit somewhat similar patterns of behaviour. Considering guanosine overrides the effect of uridine, it is not unreasonable to suspect that guanosine might be as adequate a supplement as uridine were it not for its toxic effect.

More surprisingly, the presence of orotate with guanosine seemed to cause a striking delay in the development of homozygous mutants

Table 25: Response of *pyr2-1* and *pyr2-2* to double supplementation by uridine and guanosine

Cross		$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{SM5}$ ♂								$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{mutant}$ ♂		
		Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Strain	Supple-ment (5.0×10^{-3}M)	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
pyr2-1	None	2	296	0.04	6.2	26.0±6.4	13.0±1.4	0.7	13	20	0.4	22.8±2.4
	GR	109	63	2.3	1.3	18.1±2.0	13.9±1.7	173.0	4.2	190	3.2	18.0±2.3
	GR + UR	100 (156)	274 (404)	2.1 (2.4)	5.7 (6.7)	18.1±2.0 (19.1±2.1)	15.3±2.5 (16.2±2.5)	36.5 (38.6)	2.8 (3.0)	407	8.5	18.1±2.4
	UR	402 (366)	473 (535)	8.4 (6.1)	9.9 (8.9)	16.8±2.2 (15.7±1.6)	13.4±1.8 (13.5±1.5)	85.0 (68.4)	3.4 (2.2)	529	11.0	16.2±2.0
pyr2-2	None	10	352	0.2	5.9	26.1±2.1	13.0±1.5	2.8	13.1	35	0.7	20.4±1.7
	GR	152	203	2.5	3.4	17.4±2.4	13.7±2.0	74.9	3.8	248	4.1	16.8±2.3
	GR + UR	102 (84)	274 (180)	1.7 (1.4)	4.6 (3.0)	19.5±2.8 (18.3±2.9)	16.6±3.2 (16.1±2.5)	37.2 (46.7)	2.9 (2.1)	668	11.1	17.6±2.9
	UR	386 (406)	515 (553)	6.4 (6.8)	8.6 (9.2)	16.3±2.2 (17.2±2.3)	13.5±1.9 (14.4±2.2)	75.0 (73.4)	2.7 (2.9)	1127	18.8	15.1±2.0

Numbers in parenthesis are equivalent data for the reciprocal cross (type 3 crosses)

(Table 26). For example, in type 1 crosses, *pyr2-1/pyr2-1* developmental time was 16.5 days on guanosine, 24.3 days on guanosine and orotate and 25.1 days on orotate. It should be noted that the behaviour of *pyr2-1* on guanosine in this particular experiment is somewhat different from its usual behaviour and looks as if the dose used may have been slightly higher than the usual 5.0 X 10^{-3}M. However, a comparison between the results on guanosine and orotate in this experiment and those on guanosine pooled over all experiments still exhibits the same trend, since developmental time on guanosine becomes 17.8 days. Not only were effects of orotate evident with homozygotes, but also with the heterozygous segregants, since growth of the latter was substantially improved when orotate was added to the guanosine medium, to a level characteristic of orotate alone (5.4 pfd on guanosine and orotate; 6.4 pfd on orotate and 2.0 pfd on guanosine alone). Moreover, their developmental time was also characteristic of orotate rather than guanosine (16.2 days on guansine and orotate, 14.9 days on orotate and 13.5 days on guanosine). In contrast, the effect of orotate with uridine can be described as trivial. The developmental time of homozygous mutants on uridine was 17.5 days on orotate and uridine 18.7 days and on orotate 22.7 days. That of heterozgyotes was 15.3 days, 15.2 days and 14.6 days. The average production of heterozygotes was 6.4 pfd, 4.9 pfd and 3.8 pfd. With *pyr2-2*, a similar pattern of behaviour was also observed.

These findings, together with the previous observation that of all the unphosphorylated pyrimidine precursors, orotate is the least supportive, may indicate that orotate has an active toxic effect upon the *pyr2-1* and *pyr2-2* mutants.

Table 26a: Response of *pyr2-1* and *pyr2-2* to double supplementation by orotate and guanosine

Cross		$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{SM5}$ ♂								$\frac{mutant}{mutant}$ ♀ X $\frac{mutant}{mutant}$ ♂		
		Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Strain	Supplement (5.0×10^{-3}M)	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
pyr2-1	None	10	351	0.2	5.9	21.6±1.7	13.2±1.7	2.9	8.4	10	0.2	21.3±3.0
	O^-	48	381	0.8	6.4	25.1±3.4	14.9±1.9	12.6	10.2	22	0.9	24.8±2.7
	O^- + GR	67	326	1.1	5.4	24.3±2.7	16.2±2.0	20.6	8.1	204	3.4	22.3±2.6
	GR	47	118	0.8	2.0	16.5±1.6	13.5±1.8	39.8 *	3.0	161	2.7	17.4±1.9
pyr2-2	None	5	323	0.1	6.7	20.0±3.0	13.8±2.3	1.6	6.2	17	0.3	17.6±2.4
	O^-	68	392	1.1	6.5	24.6±3.1	14.9±1.9	17.4	9.6	107	4.5	24.3±2.9
	O^- + GR	109	358	1.8	6.0	23.1±2.7	15.4±2.4	30.5	7.7	318	5.3	22.1±2.9
	GR	87	158	1.5	2.6	17.5±2.2	13.9±2.0	55.1	3.6	83	1.4	16.3±1.6

* mutant behaving as if the concentration of guanosine was higher than 5.0×10^{-3}M

Table 26b: Responses of *pyr2-1* and *pyr2-2* to double supplementation by orotate and uridine

Cross		$\frac{mutant}{mutant}$ ♀ x $\frac{mutant}{SM5}$ ♂								$\frac{mutant}{mutant}$ ♀ x $\frac{mutant}{mutant}$ ♂		
		Number of Progeny		Average Pro-duction		Developmental Time Mean ± S.D.		%Rel. Viab.	Developmental Delay (Days)	Number of Progeny	Average Pro-duction	Developmental Time Mean ± S.D.
Strain	Supplement (5.0 X 10^{-3}M)	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$	$\frac{m}{m}$	$\frac{m}{SM5}$					
pyr2-1	None	10	335	0.2	5.6	16.0±3.8	12.5±1.4	3.0	3.5	3	0.01	20.2±4.0
	O^-	15	183	0.3	3.8	22.7±5.0	14.6±1.8	8.2	8.2	35	0.6	24.6±2.8
	O^-+ UR	180	233	3.8	4.9	18.7±2.0	15.2±2.1	77.3	3.5	566	9.4	17.5±1.6
	UR	309	308	6.4	6.4	17.5±1.5	15.3±2.2	100.7	2.2	666	11.1	16.3±1.6
pyr2-2	None	5	223	0.1	6.2	19.6±3.8	14.2±1.8	2.2	5.4	17	0.3	22.4±2.7
	O^-	21	125	0.6	3.5	24.9±2.8	14.6±1.3	16.8	10.3	12	1.0	24.9±2.0
	O^-+ UR	94	82	3.9	3.4	16.7±1.3	14.5±1.3	114.6	2.2	471	9.8	16.1±1.6
	UR	165	139	6.9	5.8	15.9±1.6	15.0±2.2	118.7	1.0	362	7.5	15.9±1.5

Differential sex response of the pyrimidine requirers

It has been noted throughout the various experiments that mutant males respond better to various additives than mutant females. This effect was generally more pronounced in cases where supplementation was less adequate. Data supporting this point are reported in Table 27. Since, in general, females are known to develop faster than males, one wonders whether slow development is in some way related to better survival of the *pyr2* mutants.

Table 27: Differential sex response of *pyr2* mutants to various supplements

Strain	*pyr2-1/pyr2-1*				*pyr2-2/ pyr2-2*			
Cross	$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{SM5}$ ♂		$\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♀ x $\frac{pyr2\text{-}1}{pyr2\text{-}1}$ ♂		$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ x $\frac{pyr2\text{-}2}{SM5}$ ♂		$\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♀ x $\frac{pyr2\text{-}2}{pyr2\text{-}2}$ ♂	
Supplement (5 X 10^{-3}M)	Number of Progeny	Sex Ratio	Number of Progeny	Sex Ratio	Number of Progeny	Sex Ratio	Number of Progeny	Sex Ratio
None	61	1.2	204	0.9	73	1.0	317	1.2
CR	369	1.0	-	-	405	1.0	-	-
UR	1336	0.9	1852	0.9	1263	1.0	2172	1.2
AR+GR+UR+CR	258	1.0	-	-	362	1.2	-	-
UR+GR	100	0.8	407	1.0	102	1.1	668	0.8
UR+ O^-	180	0.8	566	0.9	94	1.2	471	1.0
HR	56	1.0	211	1.1	28	0.6	27	2.9
AR	48	1.7	131	2.1	42	1.2	26	0.9
GR	462	1.2	816	1.0	334	1.3	435	1.1
GR+O^-	67	1.4	204	0.7	109	1.0	318	1.4

Carbamyl phosphate	27	1.3	37	2.7	46	1.2	106	3.1
Carbamyl aspartate	46	1.9	82	1.0	84	1.3	172	1.4
DHO^-	47	2.4	71	1.8	68	1.0	76	1.0
O^-	103	1.1	71	1.9	134	1.0	297	1.6
U	20	1.5	44	1.0	40	0.9	416	1.7
A	6	1.0	2	∞	1	0.0	3	∞
G	5	4.0	18	2.0	0	-	0	-

Sex ratio = number of males divided by number of females

DISCUSSION

The discussion will be limited to the RNA requirers, *ade2-1*, *pyr2-1*, and *pyr2-2*. In summary, the results with these mutants are as follows:

ade2-1 is supplementable by either adenosine or inosine but not by guanosine nor by the pyrimidine ribosides, uridine and cytidine. *pyr2-1* and *pyr2-2* were found to be moderately to well supplementable by all common nucleosides, namely adenosine, guanosine, inosine, uridine and cytidine. There was little response, however, to the unribosylated bases adenine, guanine and uracil. Despite a stronger response to pyrimidine nucleosides, neither *pyr2-1* nor *pyr2-2* responded to any of the pyrimidine precursors tested. Moreover, assays of OPRTase activity showed it to be present with excess activity in the mutant (in the absence of dietary uridine) relative to the wild-type strain. In the same assay substantial levels of ODCase activity were identified in both mutant and wild-type.

With these results and the already established facts concerning nucleotide metabolism in both prokaryotic and eukaryotic organisms, it should be possible to conjecture the primary genetic lesions in these mutants.

The adenosine requirer, *ade2-1*

It is a well established fact that the purine biosynthetic pathway up to IMP formation is universal (see INTRODUCTION); this being the case, *ade2-1*, which is supplementable by inosine, should be blocked in one of the ten steps of purine biosynthesis *de novo*. Under such circumstances, adenosine and guanosine, which presumably generate adenylate (AMP) and guanylate (GMP), should also supplement. Paradoxically, however, *ade2-1* is not supplemented by guanosine. The resolution of this paradox might lie in a peculiarity of purine metabolism, since several enzymes in the guanosine salvage pathways have been reported missing in flies. In *Musca domestica* ovaries, Miller and Collins (1972) suggested the absence of GMP-reductase and guanine phosphoribosyl-transferase. Becker (1974) could not identify GMP-reductase, guanine phosphoribosyl-transferase and guanosine kinase, in fruit-fly tissue culture cells. If guanosine could not be utilized as a GMP precursor or if GMP could not be converted to AMP, then the apparently eccentric behaviour of *ade2-1* would be expected.

Unpublished studies by Johnson (pers. comm.) cast some doubts upon this satisfying resolution. She has shown that GMP-reductase exists in fruit flies, since radioactive dietary guanine is transferred into the adenylate pool, whilst both guanosine and guanine appear in the guanylate pool. Somewhat unexpectedly, guanylate derived from guanosine is not converted to adenylate. It is possible, however, that *Drosophila* S-AMP lyase is specifically inhibited by guanosine as found by Momose *et. al.* (1966-1967) in *B. subtilis*. Under these conditions, it would appear that the failure of guanosine to supplement may be a result of the failure of GMP derived from guanosine to be converted to AMP. Adenosine and inosine, on the other hand, are adequate supplements because either can be converted to both AMP and GMP.

Purine nucleoside requiring mutants (*pur1-1* and *pur1-2*) have been isolated in *D. melanogaster* (Falk, 1973). It was suggested that these mutants, too, are blocked in *de novo* purine biosynthesis. However, *pur 1* mutants do respond to both adenosine and guanosine. In view of the above discussion, this behaviour now appears paradoxical. It has been suggested by Johnson and Nash (1975) that some level of purine biosynthesis *de novo* is a prerequisite for survival in *D. melanogaster*, probably because *Drosophila* is uricotelic and as such would require purine biosynthesis *de novo* as a principal means of nitrogenous excretion (Henderson, 1972). Consequently, it is reasonable to suggest that feeding guanosine to enzymologically "leaky mutants" might divert *de novo* IMP production to the AMP branch of the pathway. The question arises as to why a similar diversion of *de novo* inosinate does not seem to occur in *ade2-1* mutants. There are several possibilities: *ade2-1* gives far fewer escapees on Sang's unsupplemented medium when compared with *pur1-1* and *pur1-2*. This may be a reflection of a lesser leak in the blocked enzyme, in which case feeding on guanosine may divert the IMP into AMP but the amount of inosinate produced would still be below the threshold required to generate enough adenylate. Alternatively, the block might be in adenylosuccinate synthetase so that despite the fact that guanosine is alleviating the burden on IMP still not enough adenylate could be produced, especially if guanosine does not generate IMP (see reference to Johnson above). When feeding on inosine, on the other hand, the high concentration of IMP would somehow override the genetic block in S-AMP-synthetase. More plausibly the mutant block could be in adenylosuccinate lyase. This enzyme catalyses both the conversion of phosphoribosyl amino-imidazole succinocarboxamide to phosphoribosyl amino-imidazole carboxamide and the conversion of adenylosuccinate (S-AMP) to adenylate (AMP), steps which occur before and after IMP in the production of AMP. The double requirement for this enzyme might magnify a quantitative (rather than absolute) reduction of its catalytic activity, yielding extremely low numbers of *ade2-1* escapees on additive-free medium, yet allowing inosine, after conversion to IMP, to supplement. At the same time the excretory function of the pathway would only be interrupted once, at the earlier stage at which the lyase is used.

Obviously, if *ade2-1* were blocked in the terminal steps of AMP production, lethality on Sang's unsupplemented medium would be the result of adenylate deficiency. If, on the other hand, the block were prior to IMP formation, the lethality would result from lack of both adenylate and guanylate. More insight into the problem may be gained by studying the supplementation pattern of increasing doses of adenosine in the presence of the presumably optimal concentration of 1.0×10^{-2}M guanosine. The fate of radioactive glycine should be followed as well as radioactive adenosine and guanosine, since the mapping showed a possible double block.

In all the above mentioned hypotheses, it was assumed that guanosine could be converted to guanylate (either directly or indirectly *via* guanine) but not to adenylate.

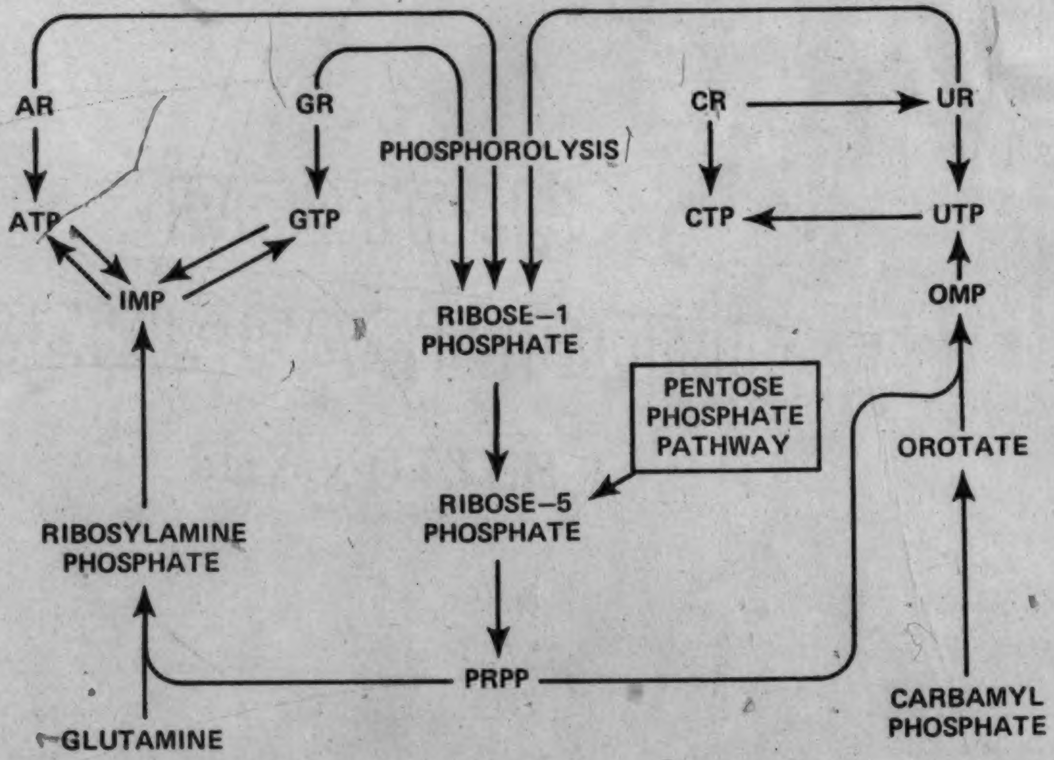
As mentioned above, Becker (1974) reported the absence of guanosine kinase and guanine phosphoribosyl-transferase, in which case the speculation concerning the diversion of inosinate into adenylate, in the presence of exogenous guanosine, would not hold. However, in addition to Johnson's unpublished results, six guanosine-requiring mutants, falling in at least three complementation groups, have been isolated so far (Falk and Nash, 1974a; Naguib and Nash (submitted for publication) and Nash (unpublished)). It does not seem plausible to assume that all these mutants utilize guanosine for some other functions than generating guanylate. It is, therefore, likely that Becker's results cannot, for some reason, be extrapolated to this system.

The pyrimidine requirers, *pyr2-1* and *pyr2-2*

These mutants respond well to pyrmidine nucleosides and rather less well to purine nucleosides. Thus, two options are available to explain their phenotype:

The first is that they must be defective in the production of a metabolite common to both purine and pyrimidine biosynthesis *de novo*. Phosphoribosyl pyrophosphate seemed to be a likely candidate as can be seen in fig. 8 . However, no mutants defective in PRPP biosynthesis have been reported in other organisms (*Escherichia coli*, Taylor, 1970; *Salmonella typhimurium*, Sanderson, 1970; *Saccharomyces cerevisciae*, Plischke *et. al.* 1975; and other systems including mammalian cells in culture, Henderson, pers. comm.). Furthermore, the absence of such mutants could be accounted for by the fact that PRPP is so central to metabolism. Indeed, Henderson, (1972) reports PRPP to be directly involved in the biosynthesis of such basic metabolites as purine, pyrimidine, nicotinate, nicotinamide, quinolinate and other ribonucleotides. In addition, except for the terminal step in PRPP biosynthesis, which is concerned with the conversion of ribose5-P to PRPP by PRPP-synthetase, several alternative biosynthetic pathways exist. A mutation in PRPP-synthetase, on the other hand, seems incompatible with the concept of conditional lethality, as this is the only known way of synthesizing PRPP. Under such circumstances it was assumed that, whether the precursors of ribose5-P were taken from Sang's medium or yeast-sucrose medium, no PRPP

Figure 8. Probable interconversions in ribonucleotide and phosphoribosyl pyrophosphate production. Many steps, particularly reverse reactions and alternative pathways, have been omitted. The nucleosides can be utilized as nucleotide precursors or phosphorolysed to yield ribosel-phosphate, which can, in turn, be converted to PRPP and used in *de novo* nucleotide production. Information gathered principally from Henderson and Patterson (1973).


AR
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PHOSPHATE
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PHOSPHATE
PATHWAY
OROTATE
RIBOSE–5
PHOSPHATE
RIBOSYLAMINE
PHOSPHATE
PRPP
GLUTAMINE
CARBAMYL
PHOSPHATE

could be made, which should result in unconditional lethality.

A second alternative is that the better response to pyrimidine ribosides relative to purine ribosides indicates a deficiency in pyrimidine biosynthesis. As this option seemed most probable, it was decided that it would be worthwhile investigating. The lack of response to pyrimidine precursors, when these have been found to supplement *rudimentary* mutants (Norby, 1970 and Falk and Nash, 1974b), suggests that, were *pyr1-1* and *pyr2-2* deficient in pyrimidine biosynthesis, the deficiency must be connected with either orotate phosphoribosyl-transferase or orotidine decarboxylase (see fig. 4). The supplementation by purine ribosides could then be explained as their being ribose-1-P donors, which would take uracil to make uridine by means of reverse phosphorolysis. Uridine is then converted into uridylate by uridine kinase. Under such circumstances no new uracil is made, which could account for the more or less inadequate supplementation by purine nucleosides. Alternatively, ribose-1-P could convert orotate to orotidine, although neither orotidine phosphorylase nor orotidine kinase is reported in the literature (Henderson and Patterson, 1973). This possibility has not been investigated in the specific case of insects so that this conjecture, at least, seemed reasonable as a last resort. However, the lack of supplementation by orotidine with both *pyr2-1* mutants and *rudimentary* mutants apparently confirmed the absence of both enzymes in *Drosophila*. This finding did not, nevertheless, disprove the possibility that the *pyr2* mutants were deficient in OPRTase and that supplementation by purine ribonucleosides resulted from the recycling of uracil. Therefore, it was decided to assay the activity of OPRTase (Shoaf and Jones, 1973). This test showed beyond any doubt the presence of both OPRTase and ODCase in *pyr2* larval extracts.

These results could still have been obtained if the mutation decreased the affinity of OPRTase to PRPP so that under physiological conditions the normal PRPP concentration in the mutants is below the concentration required for OPRTase activation; the high PRPP concentration in the assays may still have exceeded the level required by the mutant enzyme, resulting in the detection of enzyme activity. The purine riboside supplementation would remain possible because they are used directly in purine biosynthesis, thus more or less alleviating the burden on PRPP and allowing it to reach the level of concentration required for OPRTase activation. Furthermore, purine nucleosides can generate ribosel-P, which can be converted by phosphoribomutase then PRPP-synthetase to PRPP, thus again boosting the PRPP pool size to the level required for OPRTase activation. However, preliminary results investigating the dose response of OPRTase to PRPP did not support this suggestion (Nash, pers. comm.).

Ironically, enzymological characterization exhibited a relatively high activity of OPRTase in extracts from mutant larvae grown on either Sang's unsupplemented or guanosine supplemented media when compared with those from wild-type larvae grown under the same conditions or when compared with those from wild-type or mutant larvae grown on uridine. These results could be obtained if the mutation affected the regulation of OPRTase so that a higher than normal activity would result in a depletion of PRPP pool size, causing a shortage in purine ribotide synthesis or key cofactors such as nicotinamide ribonucleotide. In the presence of uridine, OPRTase activity goes back to normal thus restoring the wild-type phenotype to the *pyr2* mutants. Guanosine or adenosine, on the other hand, are sources of ribose-1-P that could be utilized directly to synthesize any required ribonucleotide *via* reverse phosphorilysis or, indirectly, to make PRPP. However, the fact that they do not affect OPRTase activity may explain their inadequacy as a supplement when compared with uridine.

Orotate may be the least adequate supplement among the pyrimidine precursors because it is a PRPP receptor, thus, if anything, contributing to the depletion of the PRPP pool. This is further illustrated by the fact that double supplementation by orotate and guanosine is parallel to the supplementation by orotate alone rather than guanosine alone, whilst the double supplementation by orotate and uridine is parallel to the supplementation by uridine rather than orotate alone. In the latter case, although orotate is a receptor of PRPP, uridine overcomes, whether directly or indirectly, the high activity of OPRTase, thus neutralizing the effect of orotate. Supplementation by both purine bases and uracil was inadequate, as would be expected if the hypothesis that PRPP level is low were correct, like orotate, they are unribosylated and act as PRPP receptors.

The same results could be also obtained if the primary lesion in the *pyr2* mutants were a defect in PRPP biosynthesis. As argued above, a total block is unthinkable because PRPP is required in the biosynthesis of numerous key metabolites. This is probably why no mutants defective in PRPP synthesis are known in bacteria, yeast or animal cell cultures. However, it is conceivable that a partial defect in PRPP production could go undetected in unicells. In contrast, in multicellular organisms, because they require highly organized spatial and temporal biosynthesis, a similar defect in a certain metabolite may be so magnified going from one new process to the other that it could lead to conditional lethality.

If, indeed, the primary lesion in *pyr2* mutants were defective PRPP biosynthesis, it would be expected and is indeed found that, in contrast to unribosylated compounds, most ribosides should supplement. Under these circumstances, increased OPRTase activity is a response to, rather than a cause of, a decreased PRPP pool size, as argued above. The question arises as to how such a response could be generated. Does a decreased endogenous PRPP concentration act directly on OPRTase or is it that other metabolites are affected that in turn affect OPRTase? Is the effect, whether direct or indirect, carried at the transcriptional, translational or enzyme level? Indeed, in bacteria starved for pyrimidines, all the enzymes of pyrimidine biosynthesis are derepressed (Yates and Pardee, 1957). In *Drosophila*, Rawls and Fristrom (1975) reported an increased activity of DHO dehase in *rudimentary* mutants. Extracts from *pyr2* mutants and wild-type flies grown on Sang's unsupplemented medium showed no significant alteration in OPRTase activity when 5.0 X 10^{-3}M uridine was added to the reaction mixture, suggesting control at the level of transcription or translation. Is the elevation of OPRTase synthesis in the *pyr2* mutants equivalent to the derepression found in microorganisms? Or is it, perhaps, inducible by an accumulation of its substrate, orotate?

Obviously, more biochemical and dietary studies are needed before a final conclusion can be reached with respect to the primary and secondary effects in the *pyr2* mutants. At the nutritional level, non-toxic ribosylated analogues, such as 6-thioguanosine, that could supply ribose but not nucleic acid bases should be tested for supplementation. Deoxribosides, that could supply nucleic acid bases but not riboses, should be tested. Double supplementation involving parallel and antiparallel dose response to a specific inhibitor of OPRTase, such as allopurinol (Fox, *et. al.* 1971) and a pyrimidine source, such as cytidine, might help resolve the question as to whether the excess enzyme level is crucial. Double supplementation with adenosine and either orotate or uridine has not been studied, perhaps they should be, in order to complete the dossier of results on responses to purine nucleosides. At the genetic level, a double mutants for *pyr2* and *suppressor of rudimentary, su(r)*, should be made and their supplementation pattern studied. The *su(r)* mutant was demonstrated by Bahn (1973) to be defective in dihydrouracil dehydrogenase and as such would accumulate uracil. The double mutant could, by accumulating uracil, generate a situation where the mutants are no longer starved for pyrimidines. Under such circumstances combined studies of dietary supplementation, enzymes of the pathway and pool size measurements may lead to an understanding of the regulation of pyrimidine biosynthesis in *Drosophila.*

At the biochemical level, a thorough investigation of OPRTase including its response to adenosine both *in vivo* and *in vitro* should be made. Activities of the other enzymes of the *de novo* pyrimidine biosynthesis should also be measured. If a positive correlation is found between the latter and OPRTase, activities of some of the enzymes involved in purine biosynthesis *de novo* should be measured. The fate of the ribose moiety in dietary nucleosides should be traced. Pool size measurements could be undertaken, although the instability of PRPP in solution makes this approach difficult. However, nucleoside pool sizes are amenable to investigation and might give some insight into the situation. If, as in bacteria, starvation for pyrimidine results in accumulation of orotate, then the orotate pool in *pyr2* and *pyr2,su(r)* may throw some light on the situation.

Rudimentary mutants show two pleiotropic effects in addition to their nutritional requirement. They are genetically rescuable female sterile and have reduced wings. The maternal effect exhibited in *pyr2* mutants is in a sense analogous to female sterility in *rudimentary* in so far as it indicated that the homzygous mutant females lay eggs which are defective. The pyrimidine mutants have normal wings, however not all *rudimentary* mutants show reduced wings.

In the context of the biochemistry and genetics of nucleotide metabolism, although not in the context of the organization of development, these effects are clearly to be considered epiphenomena.

GENERAL CONCLUSIONS

In this work, we have been concerned with gene expression and its regulation in higher eukaryotes. Our approach to the problem has been induction and characterization of genetic blocks in both purine and pyrimidine biosynthesis in *D. melanogaster*. Isolation of such mutants is in itself a scientific event of seminal importance. It is expected to parallel and extend auxotrophy in bacteria and other unicells to multicellular organisms. From a biochemical viewpoint, nucleotide metabolism can presently be investigated at the level of a whole live multicellular organism. In this context *D. melanogaster* is, for all practical purposes, a most suitable material since it is the best biologically characterized higher eukaryote. It also happens to be inexpensive and easily amenable to either genetic or biochemical analysis. As a consequence we may have provided a particularly useful system for the elucidation of nucleotide metabolism in higher organisms.

From a genetic viewpoint, organization of the genome of higher organisms as well as their gene structure can easily be analysed in this system. In fact, the close connection existing between aggregation of enzymes and gene clustering encountered in microorganisms, has also been observed in the *rudimentary* locus of *D. melanogaster*. On the other hand, because auxotrophs are conditonal lethals, they constitute a material of choice for the study of fine structure analysis of genetic loci.

With regard to the present investigation, a novel technique for the isolation of second chromosome conditional nutritional lethals, that can easily be extended to the third chromosome, has been described. Among the mutants isolated, *ade2-1* with its unorthodox response to guanosine has generated a better understanding of purine metabolism in *Drosophila*. Moreover, the unique phenotype of the *pyr2* mutants, strongly suggests that auxotrophy in higher organisms may lead to discoveries unparalleled in microorganisms. The *pyr2* mutants are of particular interest in so far as they can be an important tool in the study of the regulation of nucleotide metabolism. If they were defective in the regulation of pyrimidine biosynthesis, they will be the first true regulatory mutants in this biosynthetic pathway. If, on the contrary, they were deficient in PRPP biosynthesis they can be manipulated so as to be used in the study of the regulation of either purine or pyrimidine biosynthesis. For example, enzymological and pool level studies in double mutants of *pyr2* and *pur* mutants should lead to more insight in the regulation of nucleotide metabolism in particular, and perhaps, by extrapelation, to gene regulation in higher eukaryotes in general.

Finally, two aspects of this study, one concerned with nucleotide metabolism and the other with control of gene activity are closely associated with cancer therapy. On the other hand, nucleotides are the building blocks of nucleic acids in whose absence cell multiplication and, consequently, tumor production cannot occur. Cancerous cells, on the other hand, are described as having lost the capacity of self-regulation. Whether through further development of nucleotide metabolism as a field of study or elucidation of the general mechanism by which genes, and hence the cells, of higher organisms are regulated, it is hoped that this field of study will ultimately contribute to cancer prophylaxis.

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APPENDIX

This appendix contains computer printouts of statistics derived from experimental data produced in nutritional tests carried out. In contrast to the data presented in the preceding thesis, all flies produced under a given nutritional regime are collapsed into a single set of statistics. Data presented in any given table in the thesis proper was generally obtained from crosses reared simultaneously on a variety of media derived from the same stock medium.

The information presented in the appendix has been divided into the ten parts listed below. For the sake of completeness some items are repeated in several parts:

Part 1 Tests of all strains on yeast-sucrose, unsupplemented Sang's medium and Sang's medium supplemented with RNA

Part 2 Complementation tests

Part 3 Tests of RNA requiring strains on Sang's medium supplemented with ribosides at 5 x 10^{-3}M concentration.

Part 4 Tests of *ade2-1* on various concentrations of purine ribosides.

Part 5 Tests of *pyr2-1* and *pyr2-2* on various concentrations of adenosine, guanosine and uridine.

Part 6 Tests of *pyr2-1* and *pyr2-2* on pyrimidine precursors at 5 x 10^{-3}M concentration.

Part 7 Tests of *pyr2-1*, *pyr2-2* and *rudimentary* mutants on orotidine.

Part 8 Tests of *pyr2-1* and *pyr2-2* on uracil

Part 9 Tests of *pyr2-1* and *pyr2-2* on combinations of orotate, guanosine and uridine.

Part 10 Tests of *pyr2-1* and *pyr2-2* on various concentrations of adenosine, guanosine, inosine, adenine and guanine.

KEY TO THE APPENDIX

Each data block is identified on the left-hand side by a series of symbols; reading from left to right these represent:

The type of cross employed (single letter)

A = Heterozygous female x heterozygous male

B = Heterozygous female x homozygous male

C = Homozygous female x heterozygous male

D = Homozygous female x homozygous male

Strain identity (four symbols)

The principal symbols, A66-17, B663, B824, C210 and C426 refer to *yea8-1, yea9-1, pyr2-1, ade2-1* and *pyr2-2* as described in tests. Other symbols with one letter and three numbers are non-mutant controls.

In the complementation tests (part 2) the first and second elements of these symbols are used (i.e. A6,B6, B8, C2, C4). The first pair represents the female part, the second pair the male parent.

In part 7, *rudimentary* mutants are coded as follows $(r)^{pyr1\text{-}1}$ = 0101, $r^{pyr1\text{-}10}$ = 0110 and $r^{pyr1\text{-}19}$ = 0119. The cross in this case is as shown in the main text.

Medium (two letters)

Yeast-sucrose medium = YN

Sang's medium = SN

Supplemented Sang's medium = S-, where - indicates the nature of the supplement as follows:

A = adenosine
B = guanosine
C = uridine
D = cytidine
E = A + B + C + D
F = carbamylphosphate
G = carbamylaspartate
H = dihydroorotate
I = orotate
J = orotidine
K = guanosine + orotate
L = uridine + orotate
M = guanosine + uridine
N = no supplement
P = RNA
Q = uracil
R = inosine
S = adenine
T = guanine

Supplement concentration (three numbers)

The symbol can be transformed to a molar concentration as follows:

Symbol x y z Molar concentration = $x.y \times 10^{-Z}$

5 0 3 5.0×10^{-3}

Number of days of oviposition (two or three numbers)

Read directly

Genotype of progeny (one number)

0 = homozygote

1 = heterozygote

Sex of progeny (one number)

0 = male

1 = female

The statistics

The statistics given in order from left to right are:

Number of flies produced.

Mean number of flies produced per day of oviposition

Mean development time

Standard deviation of development time

Median development time

part 1-1

			N	PRODUCTION	DEVELOPMENT TIME		
					MEAN	SD	MEDIAN
D 8863 SS 0 110	0	0	7	0.06	15.0	1.0	15.0
		1	6	0.06	14.5	1.0	14.0
D 8863 SP 4 56	0	0	7	0.13	12.1	1.5	12.0
		1	0	0.0	999.9	999.9	999.9
D 8863 TS 3 150	0	0	296	1.97	12.1	1.2	12.0
		1	288	1.92	12.0	1.1	12.0
D C218 SS 0 363	0	0	7	0.02	25.7	1.5	26.0
		1	6	0.04	27.0	1.2	28.5
D C218 SP 4 65	0	0	393	6.05	12.3	1.6	11.5
		1	395	6.08	12.2	1.5	11.5
D C213 TS 2 150	0	0	650	4.33	11.6	1.3	10.5
		1	606	4.04	11.3	1.4	10.5
D 8824 SS 0 370	0	0	99	0.27	23.2	2.7	22.5
		1	104	0.28	24.0	2.4	23.5
D 8824 SP 4 45	0	0	456	10.13	11.8	2.1	11.5
		1	367	8.16	11.7	1.9	11.5
D 8824 TS 0 120	0	0	740	6.17	11.9	1.7	11.5
		1	673	5.61	11.8	1.7	11.5
D C126 SS 0 671	0	0	171	0.24	21.8	2.5	20.5
		1	165	0.23	23.7	2.8	22.0
D C626 SP 4 72	0	0	696	9.66	11.6	1.9	11.5
		1	627	8.71	11.5	2.0	11.0
D C626 TS 3 150	0	0	999	6.66	11.7	1.5	11.5
		1	891	5.94	11.6	1.6	11.5
D C997 SS 0 18	0	0	143	7.94	14.2	1.9	13.5
		1	138	7.67	14.2	1.7	13.5
D C957 SP 4 45	0	0	529	11.76	11.9	1.9	11.5
		1	493	10.96	11.9	1.8	11.0
D C951 TS 0 150	0	0	1128	7.51	11.9	1.4	10.5
		1	1079	7.10	11.8	1.3	10.5
D C922 SS 0 54	0	0	180	3.33	15.0	1.5	15.0
		1	175	3.24	14.7	1.4	15.0
D C922 SP 4 36	0	0	126	3.50	12.0	1.6	11.5
		1	143	3.97	12.0	1.7	11.5
D C922 TS 3 150	0	0	445	2.97	12.3	1.4	11.5
		1	385	2.57	12.1	1.5	11.5
D D151 SS 0 36	0	0	101	2.81	13.3	1.7	13.0
		1	104	2.90	12.7	1.5	13.0
D D151 SP 4 99	0	0	283	2.86	11.7	1.8	10.0
		1	278	2.81	11.1	1.6	10.0
D D151 TS 3 150	0	0	624	4.16	11.2	1.5	10.5
		1	595	3.97	10.9	1.4	10.5
D D224 SS 0 54	0	0	249	4.61	12.9	1.6	12.5
		1	269	4.98	13.0	1.5	13.0
D D224 SP 4 54	0	0	516	9.56	11.4	1.9	11.0
		1	469	8.69	11.3	1.9	11.0
D D224 TS 3 150	0	0	1176	7.84	11.2	1.7	10.5
		1	1139	7.59	11.1	1.7	10.5
D D227 SS 0 27	0	0	36	1.33	12.3	1.2	11.5
		1	39	1.44	12.4	1.2	11.5
D D227 SP 4 45	0	0	174	3.87	11.2	1.8	11.0
		1	159	3.53	11.2	1.7	11.0
D D227 TS 0 150	0	0	552	3.68	11.0	1.5	10.5
		1	600	4.00	10.8	1.5	10.5
C 6667 SS 0 177	0	0	0	0.0	999.9	999.9	999.9
		1	1	0.01	999.9	999.9	999.9
	1	0	65	0.37	11.9	1.2	11.5
		1	73	0.41	12.3	1.2	12.0
C 6667 SP 4 99	0	0	0	0.0	999.9	999.9	999.9
		1	0	0.0	999.9	999.9	999.9
	1	0	15	0.15	11.7	0.9	12.0

part 1-2

```
                          1        8      0.00     11.0      0.7     11.0

C 4467  TR   0  240 0 0          197      0.82     11.9      1.6     11.5
                      1          131      0.55     11.6      1.5     10.5
                    1 0          624      2.60     11.3      1.5     10.5
                      1          410      1.71     11.0      1.5     10.5

C 8663  SS   0  141 0 0            1      0.01    999.9    999.9    999.9
                      1            5      0.04     13.7      1.3     14.0
                    1 0          154      1.09     12.4      2.6     11.5
                      1          162      1.15     12.7      2.8     12.3

C 8663  SP   *   90 0 0            4      0.04     13.3      1.0     13.0
                      1            3      0.03     14.0      1.0     13.5
                    1 0          155      1.72     11.0      0.9     10.5
                      1          148      1.64     10.6      0.8     10.5

C 8663  TR   0  150 0 0          142      0.95     12.7      1.2     12.5
                      1          159      1.06     12.5      1.2     13.0
                    1 0          175      1.17     11.0      1.0     10.5
                      1          180      1.20     11.6      1.1     10.5

C C219  SS   0  258 0 0            2      0.01     10.0     -0.0     10.0
                      1            8      0.03     21.0      7.5     19.0
                    1 0          457      1.77     14.1      1.9     13.5
                      1          479      1.86     14.0      2.0     13.5

C C219  SP   *   90 0 0          288      3.20     11.7      1.1     11.0
                      1          279      3.10     11.6      1.0     11.0
                    1 0          295      3.28     11.2      1.0     10.5
                      1          286      3.18     11.0      1.0     11.0

C C210  TR   0  150 0 0          365      2.43     11.6      1.4     10.5
                      1          399      2.66     11.4      1.3     10.5
                    1 0          350      2.33     11.1      1.2     10.5
                      1          349      2.33     10.9      1.2     10.5

C 8824  SS   0  687 0 0           23      0.03     21.2      4.1     22.0
                      1           28      0.04     21.3      4.9     22.5
                    1 0         1787      2.60     13.1      1.6     13.5
                      1         1907      2.78     13.0      1.7     13.5

C 8824  SP   *   81 0 0          260      3.21     11.6      1.1     11.0
                      1          260      3.21     11.5      1.1     11.0
                    1 0          317      3.91     11.3      1.1     11.0
                      1          334      4.12     11.1      1.0     10.5

C 8824  TR   0  120 0 0          283      2.36     11.5      1.3     10.5
                      1          256      2.13     11.7      1.3     10.5
                    1 0          248      2.07     11.3      1.3     10.5
                      1          271      2.26     11.1      1.3     10.5
```

```
C C426  SS   0  687 0 0           41      0.06     21.9      4.9     22.5
                      1           38      0.06     22.2      5.0     24.0
                    1 0         1674      2.44     13.5      2.9     13.5
                      1         1781      2.59     13.8      2.1     13.5

C C426  SP   *   90 0 0          173      1.92     11.3      1.4     10.5
                      1          159      1.76     11.2      1.3     12.5
                    1 0          205      2.28     11.1      1.2     10.5
                      1          227      2.52     10.9      1.0     11.0

C C426  TR   0  150 0 0          371      2.47     11.2      1.2     10.5
                      1          321      2.14     10.9      1.2     10.5
                    1 0          344      2.29     11.1      1.2     10.5
                      1          349      2.33     11.1      1.2     10.5

C C951  SS   0   81 0 0          218      2.69     12.9      1.6     13.0
                      1          205      2.53     12.9      1.5     12.5
                    1 0          208      2.56     13.0      1.6     13.0
                      1          219      2.70     13.1      1.6     12.5

C C951  SP   *   90 0 0          384      4.27     10.9      1.0     10.5
                      1          371      4.12     10.6      1.1     10.5
                    1 0          397      4.41     11.5      1.6     10.5
                      1          400      4.44     11.4      1.0     11.0

C C951  TR   0  120 0 0          259      2.16     10.4      1.0     10.5
                      1          264      2.20     10.1      0.9     10.5
                    1 0          299      2.49     10.9      1.1     10.5
                      1          301      2.51     10.6      1.1     10.5

C C922  SS   0   72 0 0           90      1.25     14.7      1.4     10.5
                      1           84      1.17     14.6      1.5     13.5
                    1 0           77      1.07     12.8      1.2     12.5
                      1           78      1.08     13.2      1.3     12.5

C C922  SP   *   81 0 0          137      1.69     11.9      1.1     12.0
                      1          147      1.81     11.5      1.0     11.0
                    1 0          136      1.68     10.4      0.9     10.5
                      1          190      2.35     10.5      0.9     10.5

C C922  TR   0  108 0 0          135      1.25     11.2      1.1     10.5
                      1          114      1.06     11.0      1.0     10.5
                    1 0          167      1.55     10.6      1.1     10.5
                      1          179      1.66     10.4      1.0     10.5

C D151  SS   0   90 0 0          135      1.50     13.2      2.0     13.0
                      1          151      1.68     13.1      1.9     13.0
                    1 0          138      1.53     13.3      2.0     13.0
                      1          119      1.32     13.1      2.3     13.0

C D151  SP   *   90 0 0          246      2.73     10.7      1.0     10.5
                      1          229      2.54     10.5      1.2     10.0
```

part 1-3

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                    1 0     265      2.94      11.7      1.4      11.0
                      1     136      2.61      11.1      1.3      10.5

C D131 TR  0 150 0 0        196      1.32      10.4      1.2      10.5
                      1     222      1.48      10.3      1.3      10.5
                    1 0     301      2.01      10.4      1.3      10.5
                      1     284      1.89      10.2      1.2      10.5

C D204 SR  0  97 0 0        267      2.97      12.5      1.2      12.5
                      1     249      2.77      12.8      1.2      12.5
                    1 0     207      2.30      13.0      1.3      12.5
                      1     169      1.87      12.8      1.3      12.5

C D204 SP  4  90 0 0        341      3.79      10.9      1.0      11.0
                      1     279      3.10      10.8      0.9      10.5
                    1 0     342      3.80      11.7      1.0      11.5
                      1     392      4.36      11.0      0.9      10.5

C D204 TR  3 120 0 0        323      2.69      10.6      1.1      10.5
                      1     249      2.07      10.5      1.0       9.5
                    1 0     333      2.77      10.7      1.1      10.5
                      1     324      2.70      10.3      1.0      10.5

C D227 SR  0  81 0 0         43      0.53      13.3      1.1      12.5
                      1      43      0.53      10.8      1.5      12.5
                    1 0      46      0.57      13.0      1.2      12.5
                      1      30      0.37      13.3      2.0      12.5

C D227 SP  4  72 0 0         80      1.11      11.2      1.6      11.0
                      1      73      1.01      10.9      1.2      10.5
                    1 0     100      1.39      11.1      1.0      11.0
                      1      90      1.25      11.3      1.3      11.0

C D227 TR  0 106 0 0        106      0.98      11.9      1.2      10.5
                      1      87      0.81      10.4      1.2      10.5
                    1 0     117      1.08      11.2      1.1      11.5
                      1     114      1.06      11.0      1.4      10.5

B B663 SR  0  90 0 0         11      0.12      15.2      2.8      16.0
                      1      10      0.11      14.5      2.7      15.5
                    1 0     262      2.91      12.6      1.8      12.5
                      1     231      2.57      12.8      1.8      12.5

B B663 SP  4  93 0 0          8      9.06      13.4      0.5      13.5
                      1       0      0.0      999.9    999.9     999.9
                    1 0     376      4.10      11.1      0.9      10.5
                      1     372      4.13      10.8      0.8      10.5

B B663 TR  0 150 0 0        164      1.09      12.9      1.3      13.0
                      1     184      1.21      12.1      1.4      13.0
                    1 0     209      1.39      11.1      1.2      10.5
                      1     243      1.62      11.2      1.2      10.5
```

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B C210 SR  0 150 0 0         13      0.09      23.3      4.0      20.5
                      1      13      0.09      19.6      4.4      20.5
                    1 0     621      4.10      13.9      1.9      13.5
                      1     616      4.11      14.0      1.9      13.5

B C210 SP  4  90 0 0        405      4.50      11.4      1.1      10.5
                      1     385      4.28      11.4      1.0      10.6
                    1 0     496      5.51      11.3      1.2      11.0
                      1     458      5.09      11.0      1.1      10.5

B C210 TR  0 150 0 0        338      2.25      11.2      1.2      10.5
                      1     313      2.09      11.2      1.2      10.5
                    1 0     327      2.18      11.0      1.2      10.5
                      1     328      2.19      10.9      1.2      10.8

B M824 SR  0 183 0 0        137      0.75      20.2      2.2      20.5
                      1     114      0.62      20.6      2.5      20.5
                    1 0     565      3.09      14.0      1.7      13.5
                      1     580      3.17      14.1      1.9      13.5

B M824 SP  4  90 0 0        329      3.66      11.6      1.1      11.0
                      1     301      3.34      11.4      1.1      10.5
                    1 0     335      3.72      11.3      1.1      10.5
                      1     346      3.84      11.3      1.1      10.5

B M824 TR  0 135 0 0        300      2.22      11.3      1.3      10.5
                      1     272      2.01      11.0      1.3      10.5
                    1 0     314      2.33      11.4      1.3      10.5
                      1     310      2.30      11.2      1.4      10.5

B C426 SR  0 201 0 0        195      0.97      18.9      2.0      18.5
                      1     185      0.92      19.3      2.2      19.5
                    1 0     588      2.93      14.0      1.9      13.5
                      1     581      2.89      13.9      1.7      13.5

B C426 SP  4  90 0 0        311      3.46      11.1      1.0      10.5
                      1     283      3.14      10.9      1.0      11.0
                    1 0     335      3.72      10.9      1.0      10.5
                      1     352      3.91      11.1      1.1      10.5

B C426 TR  5 150 0 0        311      2.07      11.0      1.2      10.5
                      1     326      2.17      10.9      1.3      10.5
                    1 0     344      2.29      11.2      1.3      10.5
                      1     337      2.25      11.0      1.3      10.5

B C951 SR  0  81 0 0        188      2.32      12.8      1.3      12.5
                      1     180      2.22      12.6      1.2      12.5
                    1 0     207      2.56      12.9      1.3      12.5
                      1     212      2.62      12.9      1.3      12.5

B C951 SP  4  90 0 0        388      4.31      11.0      1.1      10.5
```

part 1-4

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                      1   309   3.43   10.9   1.3   10.5
                    1 0   435   4.83   11.7   1.4   11.5
                      1   438   4.87   11.4   1.3   11.0

B C951  TF  0  120  0 0   222   1.85   10.4   1.1   10.5
                      1   169   1.41   10.4   1.0   10.5
                    1 0   306   2.55   10.6   1.1   10.5
                      1   302   2.52   10.5   1.1   10.5

B C922  SN  3   72  0 0   156   2.17   13.7   0.9   13.5
                      1   139   1.93   13.7   1.2   13.5
                    1 0   111   1.54   12.7   1.3   12.5
                      1    93   1.29   12.7   1.0   12.5

B C922  SF  4   81  0 0   163   2.01   11.0   1.2   11.5
                      1   158   1.95   11.4   1.0   11.0
                    1 0   215   2.65   10.9   0.9   10.5
                      1   240   2.96   10.7   0.9   10.5

B C922  TF  0  120  0 0   207   1.72   11.2   1.2   11.5
                      1   183   1.52   10.8   1.1   10.5
                    1 0   288   2.40   10.9   1.2   10.5
                      1   225   1.87   10.4   1.1   10.5

B D151  SN  0   90  0 0   188   2.09   13.2   0.8   13.0
                      1   242   2.69   13.0   1.4   13.0
                    1 0   190   2.11   13.0   1.6   12.5
                      1   239   2.66   12.3   1.4   12.5

B D151  SF  4   90  0 0   318   3.53   11.0   1.2   10.5
                      1   342   3.80   10.8   1.1   10.5
                    1 0   415   4.61   11.4   1.3   11.0
                      1   439   4.88   11.3   1.2   10.8

B D151  TF  0  150  0 0   188   1.75   10.8   1.1   10.5
                      1   134   0.89   10.6   1.2   10.5
                    1 0   288   1.92   10.8   1.1   10.5
                      1   310   2.07   10.7   1.2   10.5

B D294  SN  0   90  0 0   236   2.62   13.5   1.5   12.5
                      1   217   2.41   13.4   1.6   12.5
                    1 0   202   2.24   13.3   1.4   12.5
                      1   179   1.99   13.6   1.5   12.5

B D294  SF  4   81  0 0   232   2.86   10.9   0.8   10.5
                      1   151   1.86   10.9   1.0   10.5
                    1 0   309   3.81   11.2   1.0   10.5
                      1   284   3.51   10.9   0.9   10.5

B D294  TF  0  120  0 0   247   2.06   10.4   1.1   10.5
                      1   175   1.46   10.3   1.2    9.5
                    1 0   315   2.62   10.7   1.2   10.5
                      1   303   2.53   10.4   1.1   10.5

B D227  SN  0   90  0 0    68   0.76   13.1   1.4   13.5
                      1    54   0.60   13.1   1.8   13.0
                    1 0    77   0.86   13.3   1.3   13.0
                      1    51   0.57   13.7   1.7   13.0

B D227  SF  4   72  0 0   168   2.33   11.1   1.2   10.5
                      1   141   1.96   10.8   1.3   10.5
                    1 0   224   3.11   11.4   1.2   10.5
                      1   216   3.00   11.5   1.5   10.5

B D227  TF  0  120  0 0   108   0.90   10.7   1.3   10.5
                      1   102   0.85   10.3   1.2   10.5
                    1 0   152   1.27   10.9   1.5   10.5
                      1   153   1.27   10.8   1.4   10.5

A A667  SN  0   90  0 0     0    0.0  999.9  999.9  999.9
                      1     0    0.0  999.9  999.9  999.9
                    1 0   295   3.28   13.2   1.5   12.5
                      1   263   2.92   13.4   1.7   12.5

A A667  SF  4   99  0 0     0    0.0  999.9  999.9  999.9
                      1     0    0.0  999.9  999.9  999.9
                    1 0   580   5.86   12.3   2.5   10.5
                      1   607   6.13   12.1   2.3   10.5

A A667  TF  0  240  0 0   220   0.92   12.1   1.4   12.0
                      1   125   0.52   11.9   1.3   11.5
                    1 0   667   2.78   11.0   1.5   11.5
                      1   688   2.87   11.4   1.6   10.5

A B663  SN  0  108  0 0    16   0.15   13.0   2.1   11.5
                      1    18   0.17   14.1   2.0   13.0
                    1 0   232   4.32   15.4   2.4   15.5
                      1   239   3.79   15.6   2.1   15.5

A B663  SF  4   54  0 0     4   0.07   17.0   2.9   16.5
                      1     2   0.04   12.5   3.5   12.5
                    1 0   315   5.83   11.5   1.7   10.5
                      1   330   6.11   11.2   1.7   10.5

A B663  TF  0  150  0 0   126   0.84   12.9   1.3   13.0
                      1   117   0.78   12.4   1.4   13.0
                    1 0   324   2.16   11.5   1.5   11.5
                      1   357   2.38   11.2   1.5   10.5

A C216  SN  0   36  0 0    14   0.39   13.0   1.5   14.0
                      1    40   1.11   13.5   1.4   13.0
                    1 0   262   7.28   13.8   1.7   12.5
                      1   217   6.03   14.0   1.7   14.0

A C212  SF  4   72  0 0   214   2.97   11.9   1.5   11.5
                      1   201   2.79   11.7   1.5   11.5
                    1 0   405   5.62   11.5   1.9   10.5
                      1   454   6.31   11.7   2.0   10.5
```

part 1-5

```
A  C210  TS   0   150 0 0      222      1.48     11.0     1.5     11.5
                        1      177      1.18     11.7     1.4     11.5
                      1 0      428      2.85     11.4     1.6     10.5
                        1      444      2.96     11.3     1.5     10.9

A  D024  SS   0    36 0 0       13      0.36     10.4     2.4     20.0
                        1       10      0.28     16.4     3.7     17.0
                      1 0       80      2.22     13.5     2.1     22.5
                        1       76      2.11     13.3     1.4     13.0

A  D024  SP   4    36 0 0       97      2.69     11.9     1.8     11.5
                        1       94      2.61     11.7     1.7     11.5
                      1 0      236      6.56     11.5     1.6     10.5
                        1      272      7.56     11.0     1.7     11.5

A  D024  TS   0   150 0 0      202      1.35     11.3     1.8     10.9
                        1      198      1.32     11.0     1.5     10.5
                      1 0      378      2.52     11.1     1.6     10.5
                        1      440      2.93     10.8     1.6     10.5

A  C426  SS   0    45 0 0       31      0.69     10.1     2.7     17.5
                        1       31      0.69     19.4     2.7     18.0
                      1 0      118      2.62     13.3     1.4     12.5
                        1      123      2.73     13.5     1.4     13.0

A  C426  SP   4    54 0 0      163      3.01     11.5     1.8     10.5
                        1      147      2.72     11.1     1.7     10.5
                      1 0      381      7.05     12.0     2.2     12.0
                        1      389      7.20     12.2     2.1     14.0

A  C426  TS   0   150 0 0      200      1.33     11.2     1.4     10.5
                        1      172      1.15     11.2     1.4     10.5
                      1 0      398      2.65     11.5     1.5     11.5
                        1      396      2.64     11.4     1.5     11.5

A  C951  SS   0    27 0 0       26      0.96     12.7     2.3     11.5
                        1       26      0.96     13.3     2.2     12.5
                      1 0       94      3.48     12.9     1.6     11.5
                        1       88      3.26     13.1     1.9     12.5

A  C951  SP   4    36 0 0       66      1.83     11.0     1.7     11.0
                        1       62      1.72     11.1     1.7     11.0
                      1 0      213      5.92     12.1     2.0     11.5
                        1      208      5.78     12.0     2.0     11.5

A  C951  TS   0   150 0 0      208      1.39     10.5     1.4     10.5
                        1      181      1.21     10.4     1.3      9.5
                      1 0      683      4.55     10.6     1.4     10.5
                        1      635      4.23     10.6     1.4     10.5

A  C922  SS   0    54 0 0       65      1.20     14.2     1.8     13.5
                        1      100      1.85     14.1     1.7     14.0
                      1 0      160      2.96     13.1     1.4     13.3
                        1      126      2.33     13.1     1.4     13.5

A  C922  SP   4    72 0 0       70      0.97     12.1     2.2     11.5
                        1       89      1.24     12.3     1.7     11.5
                      1 0      302      4.19     11.2     1.8     11.0
                        1      331      4.60     11.4     1.8     11.0

A  C922  TS   0   150 0 0      177      1.18     11.6     1.3     11.5
                        1      136      0.91     11.6     1.2     11.5
                      1 0      499      3.33     10.1     1.4     10.5
                        1      480      3.20     10.8     1.4     10.5

A  D151  SS   0     9 0 0        4      0.44     10.5     0.8     10.5
                        1        6      0.67     10.5     0.5     10.5
                      1 0       18      2.00     10.4     0.7     10.0
                        1        9      1.00     10.2     0.4     10.0

A  D151  SP   4    45 0 0       78      1.73     13.9     1.6     14.0
                        1       88      1.96     14.1     1.6     14.0
                      1 0      251      5.58     14.4     1.8     14.0
                        1      274      6.09     14.6     1.9     15.0

A  D151  TS   0   150 0 0       77      0.51     11.0     1.3     10.5
                        1       93      0.62     10.5     1.2     10.5
                      1 0      517      3.45     10.5     1.4      9.5
                        1      474      3.16     10.3     1.4      9.5

A  D234  SS   0     9 0 0       27      3.00     16.9     2.0     16.5
                        1       23      2.56     16.7     1.7     16.0
                      1 0       27      3.00     16.1     1.8     15.5
                        1       31      3.44     16.8     1.3     17.0

A  D234  SP   4    36 0 0       96      2.67     11.3     1.8     12.0
                        1       72      2.00     11.8     1.6     11.5
                      1 0      210      5.83     12.2     1.9     11.5
                        1      213      5.92     12.2     2.1     11.5

A  D234  TS   0   150 0 0      233      1.55     10.4     1.3     10.5
                        1      183      1.22     10.3     1.3      9.5
                      1 0      586      3.91     10.7     1.4     10.5
                        1      585      3.90     10.6     1.3     10.5

A  D227  SS   0    27 0 0       21      0.78     12.9     1.3     12.5
                        1       12      0.44     13.0     1.2     13.0
                      1 0       46      1.70     12.6     1.4     12.0
                        1       58      2.15     13.1     1.8     12.5

A  D227  SP   4    45 0 0       15      0.33     10.3     1.3      9.5
                        1       15      0.33     11.1     1.4     10.5
                      1 0      156      3.47     11.7     1.3     11.5
                        1      108      2.40     11.6     1.7     10.5
```

part 1-6

```
A  0227  28   0  150 0 0      51       0.34      10.5       1.3       10.5
                         1    50       0.33      10.1       1.2        9.5
                       1 0   381       2.54      10.8       1.5       10.5
                         1   440       2.93      10.6       1.4       10.5
```

part 2-1

```
                              DEVELOPMENT TIME
                 N  PRODUCTION   MEAN     SD   MEDIAN

C A6B6 SH 0 60 0 0    22    0.37   11.9    1.5   11.5
                 1    24    0.40   12.0    1.7   11.5
               1 0    20    0.33   11.8    1.3   11.5
                 1    16    0.27   11.5    1.3   11.5
D A6B6 SH 0 60 0 0    30    0.50   11.4    1.3
                 1    46    0.77   11.5    0.9

C A6B8 SH 0 60 0 0    26    0.43   11.6    3.1   11.5
                 1    21    0.35   12.0    4.6   11.5
               1 0    10    0.17   11.2    1.2   11.5
                 1    15    0.25   11.9    2.4   11.5
D A6B8 SH 0 60 0 0    36    0.60   12.0    2.0
                 1    50    0.83   12.0    2.4

C A6C2 SH 0 60 0 0    27    0.45   12.7    2.4   11.5
                 1    20    0.33   12.2    1.8   11.5
               1 0    17    0.28   12.1    1.1   12.5
                 1    17    0.28   12.4    1.2   12.5
D A6C2 SH 0 48 0 0    25    0.52   11.7    2.3
                 1    29    0.60   11.6    1.2

C A6C4 SH 0 60 0 0    12    0.20   11.8    1.2   11.5
                 1    16    0.27   11.4    1.8   11.5
               1 0     8    0.13   13.1    2.7   13.0
                 1    13    0.22   12.0    1.2   12.5
D A6C4 SH 0 60 0 0    21    0.35   12.1    2.2
                 1    26    0.43   11.7    2.6

C B6A6 SH 0 60 0 0    93    1.55   12.6    1.4   12.5
                 1   144    2.40   12.7    1.4   12.5
               1 0    41    0.68   12.7    1.6   12.5
                 1    69    1.15   12.5    1.9   12.5

C B6B8 SH 0 60 0 0   115    1.92   12.8    1.5   12.5
                 1   115    1.92   13.0    1.5   13.5
               1 0    61    1.02   12.8    1.8   13.5
                 1    56    0.93   12.5    2.0   12.5
D B6B8 SH 0 60 0 0   124    2.07   12.9    1.6
                 1   156    2.60   12.9    1.6

C B6C2 SH 0 60 0 0    61    1.02   12.9    1.8   13.5
                 1    82    1.37   13.2    1.8   13.5
               1 0    28    0.47   13.0    1.3   13.0
                 1    25    0.42   12.5    1.6   12.5
D B6C2 SH 0 60 0 0   142    2.37   13.0    1.5
                 1   220    3.67   13.2    1.4

C B6C4 SH 0 60 0 0    56    0.93   12.6    1.6   12.5
                 1   127    2.12   12.5    1.5   12.5
               1 0    35    0.58   12.5    1.4   12.5
                 1    62    1.03   12.4    1.5   12.5
D B6C4 SH 0 60 0 0   139    2.32   12.6    1.6
                 1   151    2.52   12.5    1.8

C B8A6 SH 0 60 0 0   131    2.18   12.9    1.6   12.5
                 1   112    1.87   12.8    1.9   13.5
               1 0    49    0.82   12.9    2.0   12.5
                 1    48    0.80   12.5    1.9   12.5

C B8B6 SH 0 60 0 0   237    3.95   13.8    1.9   13.5
                 1   213    3.55   13.4    2.0   13.5
               1 0   104    1.73   13.6    1.9   13.5
                 1    83    1.38   14.5    2.2   14.5
D B8B6 SH 0 36 0 0   215    5.97   13.7    1.5
                 1   175    4.86   13.8    1.7

C B8C2 SH 0 48 0 0    99    2.06   13.3    1.8   13.5
                 1    98    2.04   13.5    2.0   13.5
               1 0    45    0.94   13.6    1.6   13.5
                 1    47    0.98   13.4    1.9   13.5
D B8C2 SH 0 36 0 0   132    3.67   13.6    1.9
                 1   148    4.11   13.7    1.9

C B8C4 SH 0 36 0 0     4    0.11   21.8    2.4   22.5
                 1     0    0.0   999.9  999.9  999.9
               1 0    29    0.81   13.1    1.6   13.5
                 1    37    1.03   12.7    1.8   12.5
D B8C4 SH 0 60 0 0    32    0.53   22.4    2.4
                 1    12    0.20   23.9    2.4

C C2A6 SH 0 60 0 0    91    1.52   13.4    1.4   13.5
                 1    74    1.23   13.2    1.5   13.5
               1 0    75    1.25   13.2    1.8   13.5
                 1    43    0.72   11.4    1.7   13.5
```

part 2-2

```
C C2B6 SH  0   60 0 0     81     1.35    13.1    1.3    12.5
                    1    130     2.17    13.1    1.4    12.5
                  1 0     61     1.02    13.6    1.3    13.5
                    1     51     0.85    13.8    1.7    13.5
D C2B6 SH  0   60 0 0    134     2.23    12.3    1.9
                    1    122     2.03    12.5    1.5

C C2B8 SH  0   60 0 0    105     1.75    13.4    1.4    13.5
                    1    124     2.07    13.0    1.5    13.5
                  1 0     75     1.25    13.6    1.6    13.5
                    1     41     0.68    13.6    1.9    13.5
D C2B8 SH  0   60 0 0    123     2.05    13.2    1.6
                    1     95     1.58    13.2    1.4

C C2C6 SH  0   60 0 0    160     2.67    13.3    1.6    13.5
                    1    133     2.22    13.3    1.5    13.5
                  1 0     96     1.60    13.6    1.6    13.5
                    1     72     1.20    13.4    2.0    12.5

C C4A6 SH  0   36 0 0     97     2.69    14.0    1.9    14.5
                    1    106     2.94    13.9    2.1    14.5
                  1 0     48     1.33    13.1    2.2    12.5
                    1     38     1.06    13.9    1.5    13.5

D C4B6 SH  0   60 0 0    311     5.18    13.7    1.8    13.5
                    1    300     5.00    13.6    1.9    13.5

C C4B8 SH  0   60 0 0     13     0.22    22.9    2.1    22.5
                    1     13     0.22    23.7    3.8    22.5
                  1 0     70     1.17    12.8    1.7    12.5
                    1     71     1.18    12.7    1.9    12.5
D C4B8 SH  0   24 0 0      1     0.04   999.9  999.9
                    1      7     0.29    23.7    2.6

C C4C2 SH  0   60 0 0    169     2.82    13.0    1.6    13.5
                    1    153     2.55    13.2    1.6    13.5
                  1 0     62     1.03    13.1    1.8    13.0
                    1     40     0.67    12.9    1.4    13.0
D C4C2 SH  0   60 0 0    219     3.65    13.4    2.9
                    1    274     4.57    13.2    1.7

C A667 SH  0  177 0 0      0     0.0    999.9  999.9   999.9
                    1      1     0.01   999.9  999.9   999.9
                  1 0     65     0.37    11.9    1.3    11.5
                    1     73     0.41    12.3    1.2    12.0
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C 1663 SH  0  255 0 0      1     0.01   999.9  999.9   999.9
                    1      5     0.04    13.7    1.3    14.0
                  1 0    154     1.09    12.4    2.6    11.5
                    1    162     1.15    12.7    2.8    12.5

C B824 SH  0  687 0 0     33     0.05    21.2    4.1    22.5
                    1     28     0.04    21.3    4.9    23.5
                  1 0   1787     2.60    13.1    1.6    13.5
                    1   1907     2.78    13.0    1.7    13.5

C C210 SH  0  378 0 0      2     0.01    18.0    0.0    18.0
                    1      8     0.03    21.0    7.5    19.0
                  1 0    457     1.77    14.1    1.9    13.5
                    1    479     1.86    14.0    2.0    13.5

C C426 SH  0  687 0 0     41     0.06    21.9    4.0    20.5
                    1     38     0.06    22.2    5.0    23.0
                  1 0   1674     2.44    13.5    2.0    13.5
                    1   1781     2.59    13.0    2.1    13.5
```

part 3-1

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                                      DEVELOPMENT TIME
                    N   PRODUCTION   MEAN     SD     MEDIAN

C  C210  SA 503   60 0 0   38     0.63     13.2     1.9     13.5
                       1   36     0.60     12.6     2.0     12.5
                     1 0   26     0.43     12.2     1.3     12.5
                       1   16     0.27     12.7     1.6     12.8

C  C210  SB 503   60 0 0    3     0.05     13.0     4.3     10.5
                       1    4     0.07     12.3     2.4     11.5
                     1 0   42     0.70     14.2     2.0     14.5
                       1   38     0.63     14.0     1.6     14.5

C  C210  SC 503   60 0 0    3     0.05     16.0     0.6     16.5
                       1    2     0.03     17.0     1.6     17.5
                     1 0  169     2.82     14.9     1.0     15.5
                       1  177     2.95     14.9     1.8     15.5

C  C210  SD 503   60 0 0    0     0.0     999.9   999.9    999.9
                       1    0     0.0     999.9   999.9    999.9
                     1 0  123     2.05     15.0     1.7     15.5
                       1  165     2.75     15.1     2.0     15.5

C  C210  SE 503   60 0 0  117     1.95     12.6     1.9     12.5
                       1  165     2.75     12.1     1.5     11.5
                     1 0  188     3.13     12.9     2.3     13.5
                       1  233     3.88     12.6     2.2     12.5

C  C210  SF   0  258 0 0    2     0.01     18.5    -0.0     18.0
                       1    8     0.03     21.0     7.5     19.0
                     1 0  457     1.77     14.1     1.9     13.5
                       1  479     1.86     14.0     2.5     13.5

B  C210  SA 503   60 0 0   46     0.77     13.0     1.5     13.5
                       1   35     0.58     12.5     1.6     12.5
                     1 0   56     0.93     12.3     1.5     12.5
                       1   32     0.53     12.5     1.4     12.5

B  C210  SB 503   60 0 0    9     0.15     19.1     2.6     19.5
                       1   16     0.27     17.7     2.3     17.5
                     1 0   55     0.92     14.5     2.1     14.5
                       1   41     0.68     13.8     2.0     13.5

B  C210  SC 503   60 0 0    2     0.03     24.0     0.7     24.0
                       1    5     0.08     17.9     2.3     18.5
                     1 0  267     4.45     14.6     1.6     14.5
                       1  291     4.85     15.1     1.8     15.5

B  C210  SD 503   60 0 0    2     0.03     21.0     1.6     21.5
                       1    7     0.12     17.1     3.3     15.5
                     1 0  206     3.43     13.6     2.0     13.5
                       1  224     3.73     15.4     2.0     15.5
```

```
B  C210  SE 503   60 0 0  117     1.95     12.2     2.0     11.5
                       1  120     2.00     12.2     2.6     12.5
                     1 0  203     3.38     11.1     2.5     11.5
                       1  238     3.97     11.7     2.4     11.5

B  C210  SF   0  150 0 0   13     0.09     23.1     4.0     24.5
                       1   13     0.09     19.4     6.4     20.5
                     1 0  621     4.14     13.4     1.9     13.5
                       1  616     4.11     14.0     1.9     13.5

C  B824  SA 503  105 0 0   30     0.29     13.9     1.3     13.5
                       1   18     0.17     14.5     2.1     14.0
                     1 0  120     1.14     12.0     1.6     12.0
                       1  118     1.12     11.6     1.5     11.0

C  B824  SB 503  213 0 0  254     1.19     17.7     2.2     17.5
                       1  208     0.98     17.4     2.4     18.0
                     1 0  171     0.80     13.9     2.0     13.5
                       1  189     0.89     13.8     1.9     13.5

C  B824  SC 503  276 0 0  788     2.86     16.9     2.0     16.5
                       1  834     3.02     16.9     2.0     16.5
                     1 0  929     3.37     14.1     2.0     13.5
                       1  881     3.19     14.4     2.2     14.5

C  B824  SD 503   60 0 0  180     3.00     16.6     2.0     16.5
                       1  189     3.15     16.7     2.2     16.5
                     1 0  195     3.25     14.8     2.2     14.5
                       1  223     3.72     15.1     2.4     14.5

C  B824  SE 503   60 0 0  128     2.13     11.6     1.3     11.0
                       1  130     2.17     12.0     1.8     11.5
                     1 0  180     3.00     11.9     2.1     11.5
                       1  187     3.12     12.5     2.1     12.5

C  B824  SF   0  687 0 0   33     0.05     21.2     4.1     22.0
                       1   28     0.04     21.3     4.9     22.5
                     1 0 1787     2.60     13.1     1.6     13.5
                       1 1907     2.78     13.0     1.7     13.0

B  B824  SA 503  105 0 0  106     1.01     13.5     1.4     12.5
                       1   98     0.93     13.2     1.4     12.5
                     1 0  213     2.03     12.6     1.5     12.5
                       1  200     1.90     12.2     1.7     12.0

B  B824  SB 503  165 0 0  145     0.88     17.0     2.1     16.5
                       1  160     0.97     17.1     2.4     17.5
                     1 0  335     2.03     14.2     1.7     13.5
                       1  355     2.15     14.2     1.8     14.0

B  B824  SC 503  180 0 0  588     3.27     16.6     1.9     16.5
                       1  586     3.26     17.0     2.0     16.5
                     1 0  811     4.51     14.8     2.2     14.5
                       1  722     4.01     15.0     2.3     14.5
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part 3-2

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B 8826  SD 503   60 0 0     167     2.78    16.5    2.1    14.5
                      1     165     2.42    16.8    2.0    16.5
                    1 0     182     3.03    15.5    2.1    15.5
                      1     190     3.17    16.0    2.1    15.5

B 8826  SE 503   60 0 0     137     2.29    12.1    1.9    12.5
                      1     141     2.35    12.2    1.9    12.5
                    1 0     223     3.72    11.9    1.8    11.5
                      1     233     3.88    12.3    1.9    12.8

B 8826  SB   0  183 0 0     137     0.75    20.2    2.2    20.5
                      1     114     0.62    20.8    2.5    20.5
                    1 0     565     3.09    14.0    1.7    13.5
                      1     580     3.17    14.1    1.9    13.5

C C426  SA 503   80 0 0      23     0.28    14.8    2.9    14.5
                      1      19     0.23    13.0    1.8    13.5
                    1 0      62     0.78    11.1    1.5    12.5
                      1      68     0.57    11.4    1.6    10.5

C C426  SB 503  204 0 0     187     0.92    17.7    2.5    17.5
                      1     146     0.72    17.9    2.8    18.5
                    1 0     290     1.42    13.9    2.1    13.5
                      1     285     1.40    14.0    1.9    13.5

C C426  SC 503  264 0 0     763     2.89    16.3    2.0    16.5
                      1     783     2.96    16.5    2.0    16.5
                    1 0     931     3.53    14.3    2.0    14.5
                      1     926     3.51    14.6    2.1    14.5

C C426  SD 503   66 0 0     206     3.44    16.1    2.1    15.5
                      1     193     3.22    16.2    2.3    16.5
                    1 0     172     2.87    14.5    2.1    14.5
                      1     184     3.07    14.9    2.1    14.5

C C426  SE 503   60 0 0     195     3.25    12.4    2.0    12.5
                      1     166     2.77    12.2    1.9    12.5
                    1 0     207     3.45    12.9    2.4    12.9
                      1     240     4.00    12.9    2.2    12.5

C C426  SB   0  687 0 0      41     0.06    21.9    4.0    22.5
                      1      38     0.06    22.2    5.0    24.0
                    1 0    1674     2.44    13.5    2.0    13.5
                      1    1781     2.59    13.8    2.1    13.5

B C426  SA 503   60 0 0      43     0.72    11.5    1.7    13.5
                      1      30     0.50    11.7    1.5    13.5
                    1 0      93     1.55    12.9    1.8    12.5
                      1      87     1.45    12.6    1.6    12.5

B C426  SB 503  120 0 0     120     1.90    16.6    2.5    16.5
                      1     103     0.86    16.1    2.2    16.5
                    1 0     222     1.85    14.7    2.1    14.5
                      1     247     2.06    14.1    1.9    13.5
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B C426  SC 503  168 0 0     439     2.61    16.5    2.1    16.5
                      1     515     1.07    16.7    2.0    16.5
                    1 0     613     3.65    14.6    2.1    14.5
                      1     634     3.77    15.1    2.3    14.5

B C426  SE 503   60 0 0     146     2.44    17.7    2.0    17.5
                      1     173     2.89    15.5    2.2    16.5
                    1 0     198     3.30    16.4    2.7    16.5
                      1     182     3.04    16.5    2.5    16.5

B C426  SE 503   60 0 0     185     3.08    12.5    2.0    12.5
                      1     179     2.98    12.5    1.9    12.5
                    1 0     172     2.87    13.4    2.4    13.5
                      1     215     3.58    13.3    2.5    13.5

B C426  SF   0  201 0 0     195     0.97    18.9    2.0    18.5
                      1     185     0.92    19.3    2.2    19.5
                    1 0     508     2.53    14.0    1.9    13.5
                      1     581     2.89    13.9    1.7    13.5
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part 4-1

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                                            DEVELOPMENT TIME
                         N   PRODUCTION   MEAN      SD    MEDIAN

C C210  SA 103   60 0 0      1     0.02   999.9   999.9    999.9
                      1      4     0.07    16.5     3.2     16.0
                    1 0    144     2.40    12.9     1.6     13.5
                      1     99     1.65    13.1     1.6     13.5

C C210  SA 323   60 0 0     74     1.23    13.3     1.7     13.5
                      1     79     1.32    13.0     1.6     13.5
                    1 0    107     1.78    12.4     1.5     12.5
                      1     80     1.33    11.7     1.2     11.5

C C210  SA 102   60 0 0    150     2.50    12.9     1.5     13.0
                      1    149     2.48    12.6     1.5     13.5
                    1 0    116     1.93    12.7     1.5     12.5
                      1    117     1.95    12.6     1.4     12.5

C C210  SA 322   60 0 0      8     0.13    16.6     2.3     16.0
                      1     13     0.22    16.3     2.5     16.5
                    1 0     57     0.95    15.2     1.9     15.5
                      1     44     0.73    14.7     2.1     15.5

C C210  SB 103   48 0 0      0     0.0    999.9   999.9    999.9
                      1      1     0.02   999.9   999.9    999.9
                    1 0    107     2.23    14.1     2.5     14.5
                      1     72     1.50    14.0     2.0     14.5

C C210  SB 323   60 0 0      1     0.02   999.9   999.9    999.9
                      1      3     0.05    26.2     2.1     25.5
                    1 0     66     1.10    14.5     1.7     14.5
                      1     57     0.95    14.3     2.1     13.5

C C210  SB 102   60 0 0      1     0.02   999.9   999.9    999.9
                      1      2     0.03    22.0     9.9     21.5
                    1 0    167     2.78    16.1     2.0     16.5
                      1    166     2.77    15.6     2.5     15.5

C C210  SB 322   60 0 0      0     0.0    999.9   999.9    999.9
                      1      3     0.05    25.2     4.0     27.5
                    1 0     65     1.08    17.0     4.0     16.5
                      1     57     0.95    17.6     4.2     17.5

C C210  SW   3  254 0 0      2     0.01    18.0    -0.0     18.0
                      1      8     0.03    21.0     7.5     19.0
                    1 0    457     1.77    14.1     1.9     12.5
                      1    479     1.86    14.0     2.0     13.5

C C210  SB 323   48 0 0    141     2.94    12.7     1.3     12.5
                      1    113     2.35    12.7     1.6     12.5
                    1 0     91     1.90    12.9     1.6     12.5
                      1     75     1.56    12.1     1.4     11.5

C C210  SB 324   96 0 0    333     3.47    13.1     1.3     13.5
                      1    337     3.51    13.0     1.3     13.5
                    1 0    329     3.43    13.2     1.5     13.5
                      1    313     3.26    12.6     1.3     12.5

C C210  SB 322   60 0 0     10     0.17    16.5     0.7     16.5
                      1     20     0.33    16.1     1.0     16.5
                    1 0     61     1.02    14.4     1.3     14.5
                      1     45     0.75    14.6     1.3     14.5

D C210  SA 103   60 0 0     10     0.17    19.6     1.9     19.0
                      1      6     0.10    18.3     2.0     18.0

D C210  SA 323   60 0 0    174     2.90    13.0     1.8     12.5
                      1    155     2.58    12.9     1.5     12.5

D C210  SA 102   60 0 0    297     4.95    12.8     1.2     12.5
                      1    299     4.98    12.9     1.1     12.5

D C210  SA 322   60 0 0     21     0.35    15.9     1.8     16.5
                      1     27     0.45    16.2     1.3     16.5

D C210  SB 103   60 0 0      0     0.0    999.9   999.9    999.9
                      1      0     0.0    999.9   999.9    999.9

D C210  SB 323   60 0 0      0     0.0    999.9   999.9    999.9
                      1      1     0.02   999.9   999.9    999.9

D C210  SB 102   60 0 0      0     0.0    999.9   999.9    999.9
                      1      0     0.0    999.9   999.9    999.9

D C210  SB 322   60 0 0      0     0.0    999.9   999.9    999.9
                      1      0     0.0    999.9   999.9    999.9

D C210  SW   0  120 0 0      3     0.02    25.7     1.5     26.0
                      1      5     0.04    27.8     1.2     28.5

D C210  SB 103   60 0 0     15     0.25    16.0     2.5     16.5
                      1      5     0.08    16.5     1.2     16.5

D C210  SB 323   60 0 0    210     3.50    12.5     1.4     12.5
                      1    211     3.52    12.7     1.3     12.5
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part 5-1

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                          DEVELOPMENT TIME
                   N   PRODUCTION   MEAN    SD   MEDIAN

D 8824 SA 504  45 0 0     5    0.11   19.2   1.3   17.5
                    1     1    0.02  999.9 999.9  999.9

D 8824 SA 754  45 0 0     9    0.20   17.8   1.7   17.5
                    1     2    0.04   24.0   3.7   24.0

D 8824 SA 103  45 0 0     4    0.09   17.5   1.9   17.5
                    1     0    0.0   999.9 999.9  999.9

D 8824 SA 253  45 0 0    20    0.44   15.2   2.0   14.5
                    1    14    0.31   14.3   2.0   14.0

D 8824 SA 503  45 0 0    89    1.98   13.8   1.8   12.5
                    1    42    0.93   12.6   1.5   11.5

D 8824 SB 504  45 0 0    11    0.24   20.6   1.9   19.5
                    1     5    0.11   20.9   2.0   19.5

D 8824 SB 754  45 0 0     3    0.07   18.8   1.0   17.5
                    1     6    0.13   20.4   1.3   20.0

D 8824 SB 103  45 0 0    25    0.56   19.4   1.6   19.0
                    1    14    0.31   20.1   1.7   20.0

D 8824 SB 253  45 0 0    99    2.20   17.6   2.0   17.5
                    1    58    1.29   18.0   2.0   17.5

D 8824 SB 503 153 0 0   415    2.71   17.7   2.2   18.0
                    1   401    2.62   18.5   2.3   18.5

D 8824 SC 504  48 0 0   135    2.81   20.1   1.7   20.5
                    1   171    3.56   20.3   2.0   20.5

D 8824 SC 754  60 0 0   324    5.40   19.5   1.8   19.5
                    1   299    4.98   19.6   1.9   19.5

D 8824 SC 103  48 0 0   301    6.27   18.3   2.1   18.5
                    1   271    5.65   18.9   2.4   18.5

D 8824 SC 253  60 0 0   351    5.85   17.8   2.3   17.5
                    1   331    5.52   17.2   2.2   17.5

D 8824 SC 503 216 0 0  1147    5.31   16.6   1.8   16.5
                    1  1220    5.65   16.6   1.8   16.5

D 8824 SE   8 408 0 0    69    0.17   23.2   2.7   23.5
                    1   104    0.18   24.8   2.4   23.5

C 8824 SA 504  45 0 0     0    0.0   999.9 999.9  999.9
                    1     1    0.02  999.9 999.9  999.9
                  1 0    97    2.16   12.9   1.1   13.0
                    1    66    1.47   12.3   1.1   12.0

C 8824 SA 754  45 0 0     0    0.00  999.9 999.9  999.9
                    1     2    0.04   14.5   0.7   14.5
                  1 0   108    2.40   12.3   1.2   12.0
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                    1   108    2.40   12.7   1.2   12.0

C 8824 SA 103  45 0 0     2    0.04   14.0   1.4   13.5
                    1     0    0.0   999.9 999.9  999.9
                  1 0    36    0.80   12.2   1.2   12.0
                    1    25    0.56   12.1   1.2   12.0

C 8824 SA 253  45 0 0     2    0.04   17.5   2.1   17.5
                    1     0    0.0   999.9 999.9  999.9
                  1 0    28    0.62   11.5   1.2   11.5
                    1    23    0.51   11.6   1.2   12.0

C 8824 SA 503 105 0 0    30    0.29   13.9   1.7   13.5
                    1    18    0.17   14.5   2.1   14.0
                  1 0   120    1.14   12.0   1.4   12.0
                    1   118    1.12   11.6   1.5   11.0

C 8824 SB 504  45 0 0     2    0.04   20.0   0.0   20.0
                    1     0    0.0   999.9 999.9  999.9
                  1 0    69    1.53   12.8   1.1   12.0
                    1    52    1.16   12.5   1.0   13.0

C 8824 SB 754  45 0 0     3    0.07   20.0   3.0   19.5
                    1     1    0.02  999.9 999.9  999.9
                  1 0    50    1.11   12.3   1.2   14.0
                    1    68    1.51   12.0   1.2   12.0

C 8824 SB 103  45 0 0     3    0.07   16.7   0.6   17.0
                    1     2    0.04   20.5   3.5   20.5
                  1 0    22    0.49   12.8   1.1   13.0
                    1    13    0.29   11.8   1.0   12.0

C 8824 SB 253  45 0 0    39    0.87   18.1   2.4   17.5
                    1    38    0.84   19.0   1.8   20.0
                  1 0    27    0.60   12.7   1.1   13.0
                    1    24    0.53   12.5   1.1   11.5

C 8824 SB 503 213 0 0   254    1.19   17.7   2.2   17.5
                    1   208    0.98   17.9   2.4   18.0
                  1 0   171    0.80   13.9   2.0   11.5
                    1   189    0.89   13.0   1.9   13.5

C 8824 SC 504  48 0 0    22    0.46   21.9   2.3   22.0
                    1    28    0.58   20.5   2.8   20.5
                  1 0   166    3.46   12.7   1.5   12.5
                    1   192    4.00   12.5   1.5   12.5

C 8824 SC 754  36 0 0    77    2.14   19.9   3.3   20.5
                    1    41    1.14   20.6   2.1   20.5
                  1 0   176    4.89   12.9   1.9   13.5
                    1   173    4.81   12.7   1.8   12.5

C 8824 SC 103  36 0 0    70    1.94   19.9   1.9   20.5
                    1    52    1.44   23.1   2.0   20.5
                  1 0   170    4.72   12.8   1.5   12.5
                    1   177    4.92   12.8   1.5   12.5

C 8824 SC 253  60 0 0   191    3.18   17.9   2.3   19.5
                    1   183    3.05   17.9   2.5   18.5
                  1 0   197    3.28   13.2   1.3   13.5
                    1   213    3.55   13.2   1.3   13.5

C 8824 SC 503 276 0 0   788    2.86   16.9   2.0   16.5
                    1   834    3.02   16.9   2.0   16.5
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                       1 0      939     3.37    14.1     2.0    13.5
                         1      881     3.19    14.4     2.2    14.5

C 3824  SN    0  687 0 0        33     0.05    21.2     4.1    22.0
                         1       28     0.04    21.3     4.9    22.5
                       1 0     1707     2.60    13.1     1.6    13.5
                         1     1907     2.70    13.0     1.7    13.5

B 3824  SA 503  195 0 0       106     1.01    13.5     1.4    12.5
                         1       98     0.93    13.2     1.4    12.5
                       1 0      213     2.03    12.6     1.5    12.5
                         1      200     1.90    12.2     1.7    12.0

B 3824  SB 503  165 0 0       165     0.98    17.0     2.1    16.5
                         1      160     0.97    17.1     2.4    17.0
                       1 0      335     2.03    14.2     1.7    13.5
                         1      355     2.15    14.2     1.8    14.0

B 3824  SC 503  180 0 0       588     3.27    16.6     1.9    16.5
                         1      586     3.26    17.0     2.0    16.5
                       1 0      811     4.51    14.8     2.2    14.5
                         1      722     4.01    15.0     2.3    14.5

B 3824  SN    0  183 0 0       137     0.75    20.2     2.2    20.5
                         1      114     0.62    20.8     2.5    20.5
                       1 0      565     3.09    14.0     1.7    13.5
                         1      580     3.17    14.1     1.9    13.5


B C426  SA 504   36 0 0         6     0.17    18.1     2.5    17.5
                         1        3     0.08    18.4     3.2    19.5
B C426  SA 754   24 0 0        12     0.51    16.4     4.3    16.5
                         1        1     0.04   999.9   999.9   999.9
B C426  SA 103   24 0 0        11     0.47    14.3     2.7    14.5
                         1        5     0.21    15.3     0.0    15.5
B C426  SA 253   24 0 0        15     0.62    13.5     2.9    13.5
                         1        6     0.25    11.7     1.0    11.5
B C426  SA 503   12 0 0        12     1.02    15.3     4.2    15.5
                         1       14     1.15    13.4     3.4    13.5

B C426  SB 504   16 0 0         8     0.11    16.9     2.7    17.5
                         1        1     0.03   999.9   999.9   999.9
B C426  SB 754   16 0 0        12     0.34    19.8     3.0    20.5
                         1        4     0.11    19.1     2.4    19.5
B C426  SB 103   24 0 0        13     0.55    17.9     2.0    17.5
                         1        4     0.17    13.5     0.0    13.5
B C426  SF 253   16 0 0        60     1.67    17.7     2.7    17.0
                         1       18     1.07    18.8     2.8    18.5
D C426  SB 503  144 0 0       187     1.30    16.5     2.3    16.5
                         1      224     1.36    16.0     2.0    16.5
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D C426  SC 504   60 0 0       169     2.82    18.6     1.9    18.5
                         1      176     2.90    19.6     1.7    19.5
D C426  SC 754   60 0 0       379     6.32    18.4     2.1    18.5
                         1      368     6.13    18.1     2.1    19.5
D C426  SC 103   60 0 0       468     7.80    16.9     2.5    16.5
                         1      382     6.37    17.5     2.4    17.5
D C426  SC 253   60 0 0       409     6.82    15.4     2.1    14.5
                         1      442     7.37    15.9     2.2    15.5
D C426  SC 503  204 0 0      1452     7.12    15.5     2.0    15.5
                         1     1269     6.22    15.9     2.1    15.5

D C426  SN    0  471 0 0       171     0.24    21.8     3.5    20.5
                         1      145     0.20    23.7     3.8    23.0

C C426  SA 504   24 0 0         2     0.07   999.9   999.9   999.9
                         1        1     0.05   999.9   999.9   999.9
                       1 0       42     1.75    13.0     1.7    13.5
                         1       58     2.42    12.9     1.5    13.0
C C426  SA 754   48 0 0         2     0.04    14.0     2.1    14.0
                         1        2     0.05    15.7     6.6    15.5
                       1 0       42     0.87    12.5     1.5    12.5
                         1       56     1.17    13.0     1.7    12.5
C C426  SA 103   24 0 0         4     0.17    16.3     3.0    15.5
                         1        1     0.04   999.9   999.9   999.9
                       1 0       23     0.96    12.1     1.8    12.5
                         1       28     1.17    12.3     1.6    12.5
C C426  SA 253   24 0 0        25     1.04    14.4     3.7    13.5
                         1       11     0.46    15.3     3.7    15.5
                       1 0       53     2.21    12.2     2.5    11.5
                         1       54     2.25    12.2     2.1    12.5
C C426  SA 503   84 0 0        23     0.28    14.8     2.9    14.5
                         1       19     0.23    13.0     1.8    13.5
                       1 0       62     0.74    12.1     1.5    12.5
                         1       48     0.57    11.4     1.6    10.5

C C426  SB 504   48 0 0         3     0.06    17.0     5.4    19.5
                         1        3     0.07    14.4     5.4    17.5
                       1 0      117     2.44    13.1     1.6    13.5
                         1      126     2.62    13.3     1.3    13.5
C C426  SB 754   36 0 0        10     0.28    18.2     2.8    18.5
                         1        1     0.03   999.9   999.9   999.9
                       1 0       58     1.62    13.1     1.7    13.5
                         1       58     1.61    13.3     2.2    13.0
C C426  FB 103   36 0 0        13     0.37    20.5     2.7    20.5
                         1        1     0.03   999.9   999.9   999.9
                       1 0       76     2.12    13.3     1.7    13.5
                         1       84     2.34    13.1     1.6    13.5
C C426  SB 253   12 0 0        14     1.17    18.3     2.7    18.5
                         1        3     0.27    16.9     3.2    17.5
                       1 0       18     1.50    12.9     1.5    13.5
                         1       11     0.93    12.4     1.0    11.5
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C  C426  SB 503  204 0 0      187      7.92     17.7     2.5     17.5
                       1      146      0.72     17.9     2.4     18.5
                     1 0      299      1.42     13.9     2.1     13.5
                       1      285      1.40     14.0     1.9     13.5

C  C426  SC 504   60 0 0       97      1.62     22.0     2.3     21.5
                       1       74      1.23     22.7     2.5     23.5
                     1 0      175      2.92     13.4     1.8     13.5
                       1      226      3.77     13.6     1.6     13.5

C  C426  SC 754   60 0 0      140      2.33     20.2     2.3     20.5
                       1      110      1.83     20.8     2.1     20.5
                     1 0      235      3.92     12.8     1.4     12.5
                       1      234      3.90     13.1     1.4     13.5

C  C426  SC 103   60 0 0      121      2.02     19.4     2.2     19.5
                       1      135      2.25     19.6     2.1     20.5
                     1 0      199      3.32     13.7     1.4     13.5
                       1      221      3.68     13.6     1.4     13.5

C  C426  SC 253   60 0 0      165     10.08     17.7     2.2     17.5
                       1      173      2.98     17.1     2.0     17.5
                     1 0      273      4.55     13.2     1.4     13.5
                       1      280      4.67     13.6     1.6     13.5

C  C426  SC 503  264 0 0      763      2.89     16.3     2.9     16.5
                       1      780      2.96     16.5     3.0     16.5
                     1 0      931      3.53     14.3     2.0     14.5
                       1      926      3.51     14.6     2.1     14.5

C  C426  SB   0  687 0 0       41      0.06     21.9     4.0     22.5
                       1       38      0.06     22.2     5.0     24.0
                     1 0     1674      2.44     13.5     2.8     13.5
                       1     1781      2.59     13.8     3.1     13.5

B  C426  SA 503   60 0 0       43      0.72     13.5     1.7     13.5
                       1       30      0.50     11.2     1.5     13.5
                     1 0       93      1.55     12.9     1.6     12.5
                       1       87      1.45     12.6     1.6     12.5

B  C426  SA 502  120 0 0      120      1.30     16.6     2.5     16.5
                       1      103      0.86     16.3     2.2     16.5
                     1 0      222      1.85     14.2     2.1     14.5
                       1      247      2.06     14.1     1.9     13.5

B  C426  SC 503  168 0 0      435      2.61     16.5     2.1     16.5
                       1      515      3.37     16.7     2.0     16.5
                     1 0      613      3.65     14.6     2.1     14.5
                       1      634      3.77     15.1     2.3     14.5

B  C426  SB   9, 201 0 0      195      0.97     18.9     2.0     18.5
                       1      195      3.92     19.3     2.2     19.5
                     1 0      588      2.93     14.0     1.9     13.5
                       1      581      2.89     11.9     1.7     11.5
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part 6-1

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                                            DEVELOPMENT TIME
                        N    PRODUCTION    MEAN       SD      MEDIAN

D  B826  SF 503   60 0 0     27      0.45      25.6       3.5      25.5
                       1     10      0.16      25.0       1.7      25.5

D  B826  SG 503   60 0 0     41      0.68      26.6       2.1      26.5
                       1     43      0.69      27.1       2.2      27.5

D  B826  SH 503   60 0 0     46      0.77      26.0       2.3      25.5
                       1     25      0.42      28.2       1.9      28.5

D  B826  SI 503  168 0 0     44      0.26      24.6       2.7      24.5
                       1     25      0.15      25.7       2.5      25.5

D  B826  SC 503  216 0 0   1147      5.31      16.6       1.8      16.5
                       1   1220      5.65      16.6       1.8      16.5

D  B826  SN   0  588 0 0     99      0.17      23.2       2.7      22.5
                       1    104      0.18      24.0       2.4      23.5

C  B826  SF 503   60 0 0     15      0.25      21.8       4.3      20.5
                       1     12      0.20      20.6       3.6      19.5
                     1 0    108      1.80      18.8       2.2      18.5
                       1    140      2.33      19.1       2.0      19.5

C  B826  SG 503   60 0 0     30      0.50      25.4       2.7      25.5
                       1     16      0.27      26.6       3.9      27.5
                     1 0    133      2.22      15.2       1.3      14.5
                       1    140      2.33      15.4       1.4      15.5

C  B826  SH 503   60 0 0     33      0.55      27.7       2.1      25.5
                       1     14      0.23      27.2       1.5      27.5
                     1 0    208      3.47      15.6       1.7      15.5
                       1    184      3.06      16.1       2.3      15.5

C  B826  SI 503  168 0 0     53      0.32      24.9       2.8      24.5
                       1     50      0.30      25.7       4.1      26.5
                     1 0    454      2.70      15.0       1.9      14.5
                       1    477      2.84      15.4       1.9      15.5

C  B826  SC 503  276 0 0    788      2.86      16.9       2.0      16.5
                       1    834      3.02      16.9       2.0      16.5
                     1 0    929      3.37      14.1       2.0      13.5
                       1    881      3.19      14.4       2.2      14.5

C  B826  SN   0  687 0 0     33      0.05      21.2       4.1      22.0
                       1     28      0.04      21.3       4.9      22.5
                     1 0   1787      2.60      13.1       1.6      13.5
                       1   1907      2.78      13.0       1.7      13.5
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C  C426  SG 503   60 0 0     47      0.78      25.1       3.0      25.5
                       1     37      0.62      26.3       2.3      26.5
                     1 0    107      1.78      15.2       1.8      14.5
                       1    131      2.18      15.4       1.9      15.5

C  C426  SH 503   60 0 0     34      0.57      26.0       2.6      25.5
                       1     34      0.57      25.3       3.2      25.5
                     1 0    131      2.18      15.5       1.5      15.5
                       1    121      2.01      16.0       1.7      15.5

C  C426  SI 503  156 0 0     68      0.44      24.8       2.7      24.5
                       1     66      0.42      26.3       3.3      26.5
                     1 0    342      2.19      14.7       1.9      14.5
                       1    302      1.94      15.2       1.8      15.5

C  C426  SC 503  264 0 0    763      2.89      16.3       2.0      16.5
                       1    780      2.96      16.5       2.0      16.5
                     1 0    931      3.53      14.3       2.0      14.5
                       1    926      3.51      14.6       2.1      14.5

C  C426  SN   0  687 0 0     41      0.06      21.9       4.0      22.5
                       1     38      0.06      22.2       5.0      24.0
                     1 0   1674      2.44      13.5       2.0      13.5
                       1   1781      2.59      13.8       2.1      13.5

B  C426  SF 503   60 0 0     47      0.78      23.2       2.7      23.5
                       1     51      0.85      22.3       2.0      22.5
                     1 0    164      2.73      18.6       1.8      18.5
                       1    163      2.71      18.9       2.0      18.5

B  C426  SG 503   60 0 0     82      1.37      21.9       2.6      22.5
                       1     62      1.04      23.6       3.3      23.5
                     1 0    288      4.80      15.9       1.9      15.5
                       1    271      4.52      16.4       2.0      16.5

B  C426  SH 503   48 0 0     61      1.27      21.9       2.0      21.5
                       1     74      1.54      23.0       2.4      23.5
                     1 0    199      4.15      16.0       1.8      15.5
                       1    221      4.60      16.4       1.7      16.5

B  C426  SI 503   60 0 0    107      1.78      21.6       2.0      21.5
                       1     71      1.18      22.7       2.4      22.5
                     1 0    262      4.37      15.9       1.9      15.5
                       1    216      3.60      16.4       2.0      16.5

B  C426  SC 503  168 0 0    439      2.61      16.5       2.1      16.5
                       1    515      3.07      16.7       2.0      16.5
                     1 0    613      3.65      14.6       2.1      14.5
                       1    634      3.77      15.1       2.3      14.5

B  C426  SN   0  201 0 0    195      0.97      18.9       2.0      18.5
                       1    185      0.92      19.3       2.2      19.5
                     1 0    588      2.91      14.0       1.9      13.5
                       1    581      2.89      13.9       1.7      13.5
```

part 6-2

```
D DB24  SF 503   60 0 0     50     0.83    23.5    3.0    23.5
                      1     29     0.48    22.6    3.3    22.5
                    1 0    146     2.43    19.7    2.0    19.5
                      1    169     2.82    19.8    1.9    19.5

D DB24  SG 503   60 0 0     70     1.16    22.9    2.1    22.5
                      1     68     1.13    21.8    2.9    21.5
                    1 0    209     3.48    15.5    1.6    15.5
                      1    219     3.65    16.0    1.8    15.5

D DB24  SN 503   60 0 0     88     1.47    23.2    2.2    23.5
                      1     75     1.25    23.5    2.7    23.5
                    1 0    246     4.10    16.0    1.9    15.5
                      1    241     4.02    16.9    1.9    17.5

D DB24  SI 503   60 0 0    106     1.77    23.2    2.3    23.5
                      1     88     1.47    23.5    2.8    23.5
                    1 0    202     3.37    16.8    1.8    15.5
                      1    190     3.17    16.7    1.8    16.5

D DB24  SC 503  180 0 0    588     3.27    16.6    1.9    16.5
                      1    586     3.26    17.0    2.0    16.5
                    1 0    811     4.51    14.3    2.2    14.5
                      1    722     4.01    15.0    2.3    14.5

D DB24  SW   0  183 0 0    137     0.75    20.2    2.2    20.5
                      1    114     0.62    20.8    2.5    20.5
                    1 0    565     3.09    14.0    1.7    13.5
                      1    580     3.17    14.1    1.9    13.5

D C426  SF 503   60 0 0     80     1.33    25.4    2.3    25.5
                      1     26     0.43    26.8    2.4    26.5

D C426  SG 503   60 0 0     99     1.65    24.3    2.5    24.5
                      1     73     1.22    25.5    2.9    25.5

D C426  SN 503   24 0 0     38     1.58    24.8    2.2    24.5
                      1     38     1.59    26.5    2.3    26.5

D C426  SI 503  120 0 0     86     0.71    24.0    2.7    23.5
                      1     59     0.50    25.6    2.7    25.5

D C426  SC 503  204 0 0   1452     7.12    15.5    2.0    15.5
                      1   1269     6.22    15.9    2.1    15.5

D C426  SW   0  471 0 0    171     0.36    21.8    3.5    20.5
                      1    145     0.31    23.7    2.8    23.0

C C426  SF 503   60 0 0     25     0.41    24.1    3.4    24.5
                      1     21     0.35    22.8    4.3    24.5
                    1 0    118     1.97    19.3    2.0    18.5
                      1     93     1.55    19.1    2.2    18.5
```

part 7-1

```
                                              DEVELOPMENT TIME
                          N    PRODUCTION    MEAN      SD     MEDIAN

D B824  SJ 105   60 0 0        6      0.10      22.7      2.7      21.5
                      1        5      0.08      23.9      1.1      23.5

D B824  SJ 104   60 0 0        9      0.15      22.1      1.9      20.5
                      1       11      0.18      22.9      2.0      23/5

D B824  SJ 103   60 0 0       35      0.58      22.6      2.2      23.0
                      1       43      0.72      25.1      2.3      25.5

D B824  SJ 323   60 0 0        0      0.0      999.9    999.9     999.9
                      1        2      0.03      28.5      4.2      28.5

D B824  SN    0 478 0 0       99      0.17      23.2      2.7      22.5
                      1      104      0.18      24.8      2.4      23.5

C B824  SJ 105   60 0 0        2      0.03      19.0      2.1      19.0
                      1        1      0.02     999.9    999.9     999.9
                    1 0      184      3.07      12.7      1.4      12.5
                      1      198      3.30      12.5      1.4      12.5

C B824  SJ 104   60 0 0        4      0.07      26.0      1.7      26.0
                      1        0      0.0      999.9    999.9     999.9
                    1 0      264      4.40      12.6      1.5      12.5
                      1      316      5.27      12.5      1.4      12.5

C B824  SJ 103   60 0 0       12      0.20      23.0      2.7      22.0
                      1        5      0.08      26.1      0.9      25.5
                    1 0      233      3.88      13.9      1.9      13.5
                      1      202      3.37      13.8      1.9      13.5

C B824  SJ 323   60 0 0        0      0.0      999.9    999.9     999.9
                      1        0      0.0      999.9    999.9     999.9
                    1 0       32      0.53      18.2      2.7      18.5
                      1       68      1.13      17.1      2.8      17.5

C B824  SN    0 687 0 0       33      0.05      21.2      4.1      22.0
                      1       28      0.04      21.3      4.9      22.5
                    1 0     1787      2.60      13.1      1.6      13.5
                      1     1907      2.78      13.0      1.7      13.5

D C426  SJ 105   60 0 0        6      0.10      28.5      1.9      28.5
                      1       10      0.17      29.8      1.8      29.0

D C426  SJ 104   60 0 0       32      0.53      28.1      1.9      28.5
                      1       24      0.40      29.0      1.7      29.0

D C426  SJ 103   60 0 0        4      0.07      25.0      1.3      25.0
                      1        7      0.12      27.5      1.8      28.5

D C426  SJ 323   60 0 0        0      0.0      999.9    999.9     999.9
                      1        0      0.0      999.9    999.9     999.9

D C426  SN    0 471 0 0      171      0.24      21.8      3.5      20.5
                      1      165      0.20      23.7      2.8      23.0


C C426  SJ 105   60 0 0        2      0.03      27.0      2.1      26.5
                      1        3      0.05      27.8      1.2      25.5
                    1 0      184      3.07      13.2      1.5      13.5
                      1      162      2.70      13.5      1.6      13.5

C C426  SJ 104   60 0 0        5      0.08      27.9      1.1      27.5
                      1       11      0.18      27.7      1.9      27.5
                    1 0      227      3.78      13.1      1.4      12.5
                      1      167      2.78      13.2      1.3      13.5

C C426  SJ 103   60 0 0        1      0.02     999.9    999.9     999.9
                      1        2      0.03      29.0      2.1      29.0
                    1 0      136      2.27      14.8      2.1      14.5
                      1      136      2.27      14.8      2.3      14.5

C C426  SJ 323   60 0 0        0      0.0      999.9    999.9     999.9
                      1        0      0.0      999.9    999.9     999.9
                    1 0        6      0.10      18.5      1.5      19.0
                      1       16      0.27      17.8      1.5      18.0

C C426  SN    0 687 0 0       41      0.06      21.9      4.0      22.5
                      1       38      0.06      22.2      5.8      24.0
                    1 0     1674      2.44      13.5      2.0      13.5
                      1     1781      2.59      13.8      2.1      13.5

D 0101  SJ 133  240 0 0      120      0.50      17.9      2.2      17.5
                      1      400      1.67      16.3      2.4      16.5

D 0101  SN    0 240 0 0       31      0.13      16.9      1.9      16.5
                      1      364      1.52      14.5      1.4      14.5

D 0110  SJ 133  240 0 0        0      0.0      999.9    999.9     999.9
                      1      182      0.76      18.5      1.8      18.5

D 0110  SN    0 240 0 0        0      0.0      999.9    999.9     999.9
                      1      360      1.50      15.5      1.6      15.5

D 0119  SJ 133  240 0 0        0      0.0      999.9    999.9     999.9
                      1      194      0.81      15.9      1.8      15.5

D 0119  SN    0 240 0 0        0      0.0      999.9    999.9     999.9
                      1      188      0.78      14.3      1.4      14.5
```

part 8-1

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                                                DEVELOPMENT TIME
                          N     PRODUCTION    MEAN       SD     MEDIAN

D  B824  SQ 503   60 0 0     22      0.36      23.6      2.9      23.5
                       1     22      0.36      24.8      2.5      23.5

D  B824  SC 503  216 0 0   1147      5.31      16.6      1.8      16.5
                       1   1220      5.65      16.6      1.8      16.5

D  B824  SW   0  591 0 0     99      0.17      23.2      2.7      22.5
                       1    104      0.18      24.8      2.4      23.5

C  B824  SQ 503   60 0 0     24      0.40      22.8      2.9      23.5
                       1     12      0.20      22.7      2.6      23.5
                     1 0    296      4.93      14.5      2.3      13.5
                       1    299      4.98      14.6      2.3      14.5

C  B824  SC 503  276 0 0    788      2.86      16.9      2.0      16.5
                       1    834      3.02      16.9      2.0      16.5
                     1 0    929      3.37      14.1      2.0      13.5
                       1    881      3.19      14.4      2.2      14.5

C  B824  SW   0  687 0 0     33      0.05      21.2      4.1      22.0
                       1     28      0.04      21.3      4.9      22.5
                     1 0   1787      2.60      13.1      1.6      13.5
                       1   1907      2.78      13.6      1.7      13.5

D  C426  SQ 503   60 0 0    261      4.35      20.2      2.5      20.5
                       1    155      2.58      21.5      2.7      21.5

D  C426  SC 503  204 0 0   1452      7.12      15.5      2.0      15.5
                       1   1269      6.22      15.9      2.1      15.5

D  C426  SW   0  471 0 0    171      0.36      21.8      3.3      20.5
                       1    145      0.30      23.7      2.8      23.0

C  C426  SQ 503   60 0 0     19      0.32      25.2      2.2      25.5
                       1     21      0.35      23.4      3.2      24.5
                     1 0    221      3.68      13.9      1.5      13.5
                       1    247      4.12      14.6      1.6      14.5

   [illegible]426  SC 503  264 0 0    763      2.89      16.3      2.0      16.5
                       1    780      2.96      16.5      2.0      16.5
                     1 0    931      3.53      14.3      2.0      14.5
                       1    926      3.51      14.6      2.1      14.5

C  C426  SW   0  687 0 0     41      0.06      21.9      4.9      22.5
                       1     38      0.06      22.3      5.9      24.0
                     1 0   1674      2.44      13.5      2.0      13.5
                       1   1781      2.59      13.8      2.1      13.5
```

part 9-1

					DEVELOPMENT TIME		
			N	PRODUCTION	MEAN	SD	MEDIAN
D B824 SB 503 153	0	0	415	2.71	17.7	2.2	18.0
		1	401	2.62	18.5	2.3	18.5
D B824 SE 503 60	0	0	85	1.42	22.3	2.8	22.5
		1	119	1.98	22.3	2.5	22.5
D B824 SC 503 216	0	0	1187	5.31	16.6	1.8	16.5
		1	1220	5.65	16.6	1.8	16.5
D B824 SL 503 60	0	0	266	4.43	17.7	1.6	17.5
		1	300	5.00	17.4	1.5	17.5
D B824 SB 503 48	0	0	202	4.21	18.3	2.5	18.5
		1	206	4.29	17.8	2.3	18.5
D B824 SN 0 498	0	0	99	0.17	23.2	2.7	22.5
		1	104	0.18	24.8	2.4	23.5
C B824 SB 503 213	0	0	254	1.19	17.7	2.2	17.5
		1	208	0.98	17.9	2.4	18.0
	1	0	171	0.80	13.9	2.0	13.5
		1	169	0.89	13.8	1.9	13.5
C B824 SK 503 60	0	0	33	0.65	23.1	2.6	22.5
		1	28	0.47	23.4	3.0	23.5
	1	0	149	2.48	15.3	2.1	15.5
		1	177	2.95	15.1	1.9	14.8
C B824 SC 503 276	0	0	788	2.86	16.9	2.0	16.5
		1	834	3.02	16.9	2.0	16.5
	1	0	929	3.37	14.1	2.0	13.5
		1	881	3.19	14.4	2.2	14.5
C B824 SL 503 48	0	0	81	1.69	17.4	1.7	17.5
		1	99	2.06	17.9	2.3	17.8
	1	0	121	2.52	14.1	2.2	14.5
		1	113	2.35	14.4	2.3	13.8
C B824 SR 503 48	0	0	43	0.90	18.3	2.0	18.5
		1	57	1.19	18.0	2.0	18.5
	1	0	133	2.77	15.4	2.6	15.5
		1	141	2.94	15.7	2.3	14.5
C B824 SN 0 487	0	0	33	0.05	21.2	4.1	22.0
		1	28	0.04	21.3	4.9	22.5
	1	0	1787	2.60	13.1	1.6	13.5
		1	1307	2.78	13.0	1.7	13.5
D B824 SB 503 145	0	0	145	[illegible]	17.6	2.1	16.5
		1	160	0.97	17.1	2.4	17.0
	1	0	335	2.03	14.2	1.7	13.5
		1	355	2.15	14.2	1.8	14.0
D B824 SC 503 180	0	0	488	1.27	16.6	1.9	16.5
		1	586	3.26	17.0	2.0	16.5
	1	0	811	4.51	14.8	2.2	14.5
		1	722	4.01	15.0	2.3	14.5
D B824 SR 503 60	0	0	72	1.20	19.5	2.3	19.5
		1	84	1.40	18.8	1.9	19.0
	1	0	194	3.23	16.1	2.4	15.5
		1	210	3.50	16.3	2.6	15.5
D B824 SE 0 183	0	0	137	0.75	20.7	2.2	20.5
		1	114	0.62	20.8	2.5	20.5
	1	0	565	3.09	14.0	1.7	13.5
		1	580	3.17	14.1	1.9	13.5
D C426 SB 503 144	0	0	187	1.30	16.5	2.3	16.5
		1	224	1.56	16.8	2.8	16.5
D C426 SK 503 60	0	0	183	3.05	21.6	2.6	21.5
		1	135	2.25	22.7	3.2	22.5
D C426 SC 503 204	0	0	1452	7.12	15.5	2.0	15.5
		1	1269	6.22	15.9	2.1	15.5
D C426 SL 503 24	0	0	240	10.00	16.3	1.5	16.5
		1	231	9.62	15.8	1.6	15.5
D C426 SB 503 60	0	0	297	4.95	17.8	2.9	18.5
		1	371	6.18	17.5	2.9	17.5
D C426 SN 0 471	0	0	171	0.24	21.8	3.5	20.5
		1	145	0.20	23.7	2.8	23.0
C C426 SB 503 204	0	0	187	0.92	17.7	2.5	17.5
		1	146	0.72	17.9	2.4	18.5
	1	0	290	1.42	13.9	2.1	13.5
		1	285	1.40	14.0	1.9	13.5
C C426 SK 503 60	0	0	55	0.91	23.0	2.9	22.5
		1	54	0.90	23.3	2.8	23.5
	1	0	173	2.88	15.9	2.4	15.5
		1	184	3.06	15.4	2.3	15.5
C C426 SC 503 264	0	0	763	2.89	16.1	2.0	16.5
		1	780	2.96	16.5	2.0	16.5
	1	0	931	3.53	14.3	2.0	14.5
		1	926	3.51	14.6	2.1	14.5

part 9-2

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C  C426  SL 503   24 0 0      51      2.12    17.0    1.4    16.5
                       1      42      1.75    16.2    1.2    16.5
                     1 0      39      1.62    14.3    1.2    14.5
                       1      43      1.79    14.6    1.3    14.5

C  C426  SB 503   60 0 0      53      0.88    20.0    3.0    20.5
                       1      49      0.81    19.0    2.4    18.5
                     1 0     152      2.53    17.0    3.4    16.5
                       1     122      2.03    16.1    2.4    16.5

C  C426  SM   0  687 0 0      41      0.06    21.9    4.0    22.5
                       1      38      0.06    22.2    5.0    24.0
                     1 0    1674      2.44    13.5    2.0    13.5
                       1    1781      2.59    13.8    2.1    13.8

B  C426  SS 503  120 0 0     120      1.00    16.6    2.5    16.5
                       1     103      0.86    16.3    2.2    16.5
                     1 0     227      1.85    14.2    2.1    14.5
                       1     247      2.06    14.1    1.9    13.5

B  C426  SC 503  168 0 0     439      2.61    16.5    2.1    16.5
                       1     515      3.07    16.7    2.0    16.5
                     1 0     613      3.65    14.6    2.1    14.5
                       1     634      3.77    15.1    2.3    14.5

B  C426  SB 503   60 0 0      34      0.57    19.0    3.3    19.5
                       1      50      0.83    17.8    2.5    18.5
                     1 0      91      1.52    16.6    2.9    15.5
                       1      89      1.48    15.7    2.1    15.5

B  C426  SX   0  201 0 0     195      0.97    18.9    2.0    18.5
                       1     185      0.92    19.3    2.2    19.5
                     1 0     588      2.93    14.0    1.9    13.5
                       1     561      2.89    13.9    1.7    13.5
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Part 10-1

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                                       DEVELOPMENT TIME
                         N    PRODUCTION    MEAN        SD

C  B824  SA   102  45 1 1   68     1.51      11.8       1.2
                        0   72     1.60      12.4       1.4
                      0 1   79     1.76      13.7       1.1
                        0   65     1.44      14.1       1.1

D  B824  SA   102  45 0 1  133     2.96      13.2       1.3
                        0  124     2.76      13.6       1.3

C  B824  SA   502  45 1 1   28     0.62      17.9       1.9
                        0   10     0.22      18.2       2.2
                      0 1    2     0.04      22.0       0.0
                        0    1     0.02     999.9     999.9

D  B824  SA   502  45 0 1    5     0.11      21.6       1.3
                        0    8     0.18      21.4       1.3

C  B824  SB   102  36 1 1  131     3.64      14.3       1.8
                        0   83     2.31      14.2       2.0
                      0 1   28     0.78      19.3       2.0
                        0   10     0.28      20.7       1.7

D  B824  SD   102  45 0 1  137     3.04      18.8       2.0
                        0  101     2.24      17.9       1.8

C  B824  SD   502  45 1 1    0     0.00     999.9     999.9
                        0    0     0.00     999.9     999.9
                      0 1    0     0.00     999.9     999.9
                        0    0     0.00     999.9     999.9

D  B824  SD   502  45 0 1    0     0.00     999.9     999.9
                        0    1     0.02     999.9     999.9

C  B824  SR   503  60 1 1   61     1.02      13.0       1.4
                        0   53     0.88      13.2       1.4
                      0 1   28     0.47      14.5       1.4
                        0   28     0.47      14.4       1.2

D  B824  SR   503  60 0 1  102     1. 3      14.1       1.9
                        0  109     1.82      14.0       1.9

C  B824  SS   103  48 1 1   60     1.25      12.7       1.4
                        0   45     0.94      12.6       1.4
                      0 1    0     0.00     999.9     999.9
                        0    0     0.00     999.9     999.9

D  B824  SS   103  48 0 1    1     0.02     999.9     999.9
                        0    1     0.02     999.9     999.9

C  B824  SS   503  48 1 1   17     0.35      13.0       1.9
                        0   14     0.29      12.1       0.9
                      0 1    3     0.06      16.5       2.6
                        0    3     0.06      17.8       2.9

D  B824  SS   503  48 0 1    0     0.00     999.9     999.9
                        0    2     0.04      26.5       0.0

C  B824  ST   103  48 1 1  206     4.29      15.8       2.9
                        0  190     3.96      15.1       1.7
                      0 1    1     0.02     999.9     999.9
                        0    1     0.02     999.9     999.9

D  B824  ST   103  48 0 1   16     0.33      25.8       3.3
                        0    9     0.19      21.5       2.3

C  B824  ST   503  48 1 1  160     3.33      14.8       1.4
                        0  161     3.35      14.9       1.6
                      0 1    1     0.02     999.9     999.9
                        0    4     0.08      24.3       0.5

D  B824  ST   503  48 0 1    6     0.13      24.2       3.5
                        0   12     0.25      24.0       1.9

C  B824  SN   000 687 1 1 1907     2.78      13.0       1.7
                        0 1787     2.60      13.1       1.6
                      0 1   28     0.04      21.3       4.9
                        0   33     0.05      21.2       4.1

D  B824  SN   000 498 0 1  104     0.17      24.8       2.4
                        0   99     0.16      23.2       2.7

C  C426  SA   102  45 1 1   30     0.67      11.7       1.1
                        0   35     0.78      12.0       1.2
                      0 1   17     0.38      14.0       1.1
                        0   20     0.44      14.7       2.0

D  C426  SA   102  45 0 1   39     0.87      14.8       1.7
                        0   47     1.04      14.6       1.7

C  C426  SA   502  45 1 1    2     0.04      18.5       1.4
                        0    4     0.09      17.8       1.3
                      0 1    0     0.00     999.9     999.9
                        0    0     0.00     999.9     999.9

D  C426  SA   502  45 0 1    0     0.00     999.9     999.9
                        0    0     0.00     999.9     999.9

C  C426  SD   102  45 1 1   63     1.40      13.9       1.5
                        0   48     1.07      14.1       1.7
                      0 1   20     0.44      18.8       2.2
                        0   21     0.47      19.1       2.1

D  C426  SD   102  45 0 1   40     0.89      19.7       1.9
                        0   71     1.58      19.4       2.3

C  C426  SD   502  45 1 1    1     0.02     999.9     999.9
                        0    1     0.02     999.9     999.9
                      0 1    1     0.02     999.9     999.9
                        0    0     0.00     999.9     999.9

D  C426  SD   502  45 0 1    1     0.02     999.9     999.9
                        0    6     0.13      21.0       1.1

C  C426  SR   503  60 1 1   17     0.28      12.3       1.5
                        0   34     0.57      12.5       1.6
                      0 1    7     0.12      15.2       2.0
                        0   10     0.17      13.9       0.8

D  C426  SR   503  48 0 1    7     0.15      15.6       2.0
                        0   20     0.42      14.9       1.5

C  C426  SS   103  36 1 1   16     0.44      12.3       1.6
                        0   18     0.50      12.7       2.0
                      0 1    0     0.00     999.9     999.9
                        0    0     0.00     999.9     999.9

D  C426  SS   103  48 0 1    1     0.02     999.9     999.9
                      0 0    0     0.00     999.9     999.9
```

Part 10-2

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C  C426  SS   503   48 1 1       7         0.15     14.2      2.4
                         0      21         0.44     13.7      1.7
                       0 1       1         0.02    999.9    999.9
                         0       0         0.00    999.9    999.9

D  C426  SS   503   48 0 1       0         0.00    999.9    999.9
                         0       3         0.06     26.5      2.8

C  C426  ST   103   48 1 1      75         1.56     14.5      2.2
                         0      58         1.21     14.3      1.9
                       0 1       0         0.00    999.9    999.9
                         0       1         0.02    999.9    999.9

D  C426  ST   103   48 0 1       0         0.00    999.9    999.9
                         0       2         0.04     30.5      0.0

C  C426  ST   103   48 1 1      87         1.81     16.5      1.7
                         0      77         1.60     16.4      1.6
                       0 1       0         0.00    999.9    999.9
                         0       0         0.00    999.9    999.9

D  C426  ST   503   48 0 1       0         0.00    999.9    999.9
                         0       0         0.00    999.9    999.9

C  C426  SH   000  687 1 1    1781         2.59     13.7      2.1
                         0    1674         2.44     13.5      2.0
                       0 1      38         0.06     22.2      5.0
                         0      41         0.06     21.9      4.0

D  C426  SH   000  471 0 1     145         0.20     23.7      2.8
                         0     171         0.24     21.8      3.5
```